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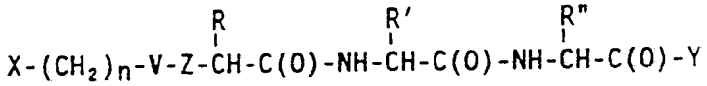

(1)

(57) Abstract

The subject invention involves anti-inflammatory compounds having structure (1), wherein (a) n is an integer of from 0 to about 2; (b) -R is selected from straight or branched alkyl, saturated or unsaturated with 1 or 2 double bonds, having from 1 to about 6 carbon atoms; and cyclic alkyl, saturated or unsaturated with 1 or 2 double bonds, having from 3 to about 13 carbon atoms; and the carbon atom to which -R is bonded is in either D or L configuration; (c) -R′ is selected from branched alkyl, saturated or unsaturated with 1 or 2 double bonds, having from 3 to about 6 carbon atoms; cyclic alkyl, saturated or unsaturated with 1 or 2 double bonds, having from 3 to about 13 carbon atoms; and arylalkyl, the alleyl portion being saturated and having from 1 to about 3 carbon atoms; and the carbon atom to which -R′ is bonded is in L configuration; (d) -R″ is $-(CH_2)_m$-A-NH_2 or $-(CH_2)_m$-A-B-$C(NH_2)=NH$, wherein m is an integer of from 1 to about 5; -A- is a covalent bond, or p-phenyl or p-cyclohexyl; and -B- is a covalent bond or -NH-; and the carbon atom to which -R″ is bonded is in L configuration; (e) -Y is hydrogen or trifluoromethyl; (f) -Z- is -O- or -NH-; (g) -V- is selected from -OC(O)-, -N(Q)C(O)-, -N(Q)C(S)-, -C(O)-, $-SO_2$- and -P(O)(OH)-; when -V- is -OC(O)-, -Z- is -NH-; (h) -X is selected from the group consisting of cyclic alkyl, branched alkyl having at least two branches, and aryl, each having from 5 to about 20 carbon atoms; and (i) -Q is selected from the group consisting of hydrogen; and straight or branched chain alkyl, saturated or unsaturated with 1 or 2 double bonds, having from 1 to about 6 carbon atoms; or -Q and -X are covalently linked forming a cyclic moiety which includes the nitrogen to which -Q is bonded and from 5 to about 20 carbon atoms. The subject invention also involves pharmaceutical compositions comprising the above compounds, and methods for treating inflammation or pain using such compounds and compositions.

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TRIPEPTIDE DERIVATIVE ANTI-INFLAMMATORY AGENTS

TECHNICAL FIELD

The subject invention relates to novel tripeptide derivatives which are useful as anti-inflammatory agents.

BACKGROUND OF THE INVENTION

Certain tripeptide derivatives having various biological activities are known. The following references disclose such tripeptide derivatives:

U.S. Patent No. 4,242,329 issued to Claeson, Simonsson & Arielly on December 30, 1980; U.S. Patent No. 4,316,889 issued to Bajusz, Hasenohrl, Barabas & Bagdy on February 23, 1982; U.S. Patent No. 4,399,065 issued to Bajusz, Hasenohrl, Barabas & Bagdy on August 16, 1983; U.S. Patent No. 4,401,594 issued to Umezawa, Takeuchi, Aoyagi, Ishii, Saino & Someno on August 30, 1983; U.S. Patent No. 4,478,745 issued to Bajusz, Hasenohrl, Barabas & Bagdy on October 23, 1984; U.S. Patent No. 4,528,133 issued to Kasafirek, Fric, Slaby & Robalova on July 9, 1985; U.S. Patent No. 4,596,789 issued to Dutta, Stein, Trainor & Wildonger on June 24, 1986; U.S. Patent No. 4,623,639 issued to Hassall, Johnson & Roberts on November 18, 1986; U.S. Patent No. 4,703,036 issued to Bajusz, Hasenohrl, Bagdy, Barabas, Dioszegi, Fittler, Jozsa, Horvath & Jozst on October 27, 1987; U.S. Patent No. 4,880,780 issued to Trainor & Stein on November 14, 1989; U.S. Patent No. 4,883,863 issued to Abe, Yaginuma, Nagasawa & Kuroiwa on November 28, 1989; U.S. Patent No. 4,902,781 issued to Mizoue, Okazaki, Hanada, Omura & Amamoto on February 20, 1990; PCT Patent Application No. 84/00365 of Galpin & Wilby, published February 2, 1984; Japanese Patent Application No. 47-30618 of Toray Inds. Inc., published November 9, 1972; Japanese Patent Application No. 57-145846 of Mitsubishi Chem. Ind. KK, published July 19, 1974; Japanese Patent Application No. 58-164563 of Daiichi Kagaku Yaku, published March 25, 1982; Aoyagi, Miyata, Nanbo, Kojima, Matsuzaki, Ishizuka, Takeuchi & Umezawa, "Biological Activities of Leupeptins", The Journal of Antibiotics, Vol. XXII, No. 11 (Nov. 1969), pp. 558-568; Bajusz, Barabas, Tolnay, Szell & Bagdy,

"Inhibition of Thrombin and Trypsin by Tripeptide Aldehydes", Int. J. Peptide Protein Res., Vol. 12 (1978), pp. 217-221; Gaal, Bacsy & Rappay, "Cationic Ferritin Uptake by Cultured Anterior Pituitary Cells Treated with the Proteinase Inhibitor, BOC-DPhe-Phe-Lys-H", Histochemistry, Vol. 88 (1988), pp. 401-406; Makara, Rappay, Garamvolgyi, Nagy, Danko & Bajusz, "The Tripeptide Aldehyde, Boc-DPhe-Phe-Lysinal, is a Novel Ca^{2+} Channel Inhibitor in Pituitary Cells", European Journal of Pharmacology, Vol. 151 (1988), pp. 147-149; Nagy, Makara, Horvath, Rappay, Kurcz & Bajusz, "Tripeptide Aldehyde Protease Inhibitors May Depress in Vitro Prolactin and Growth Hormone Release", Endocrinology, Vol. 116, No. 4 (1895), pp. 1426-1432; Rappay, Makara, Bajusz & Nagy, "Various Proteinase Inhibitors Decrease Prolactin and Growth Hormone Release by Anterior Pituitary Cells", Life Sciences, Vol. 36 (1985), pp. 549-555.

Some of the above references disclose tripeptide derivatives having anti-inflammatory activity.

It is an object of the subject invention to provide novel tripeptide derivatives which are useful as anti-inflammatory agents.

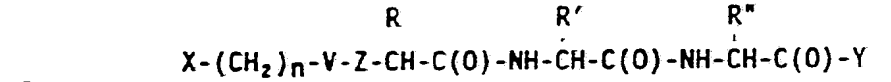
It is a further object of the subject invention to provide novel tripeptide derivatives which reduce the bone resorption and/or heterotopic bone formation associated with rheumatoid arthritis.

It is also an object of the subject invention to provide novel pharmaceutical compositions comprising certain tripeptide derivatives.

It is also an object of the subject invention to provide methods for treating diseases characterized by inflammation.

SUMMARY OF THE INVENTION

The subject invention involves anti-inflammatory compounds having the following structure:



wherein

(a) n is an integer of from 0 to about 2;

(b) -R is selected from straight or branched alkyl, saturated or unsaturated with 1 or 2 double bonds, having from 1 to about 6 carbon atoms; and cyclic alkyl, saturated or unsaturated with 1 or 2 double bonds, having from 3 to about 13 carbon atoms; and the carbon atom to which -R is bonded is in either D or L configuration;

(c) -R′ is selected from branched alkyl, saturated or unsaturated with 1 or 2 double bonds, having from 3 to about 6 carbon atoms; cyclic alkyl, saturated or unsaturated with 1 or 2 double bonds, having from 3 to about 13 carbon atoms; and arylalkyl, the alkyl portion being saturated and having from 1 to about 3 carbon atoms; and the carbon atom to which -R′ is bonded is in L configuration;

(d) -R" is $-(CH_2)_m$-A-NH_2 or $-(CH_2)_m$-A-B-$C(NH_2)$=NH, wherein m is an integer of from 1 to about 5; -A- is a covalent bond, p-phenyl or p-cyclohexyl; and -B- is a covalent bond or -NH-; and the carbon atom to which -R" is bonded is in L configuration;

(e) -Y is hydrogen or trifluoromethyl;

(f) -Z- is -O- or -NH-;

(g) -V- is selected from -OC(O)-, -N(Q)C(O)-, -N(Q)C(S)-, -C(O)-, $-SO_2$- and -P(O)(OH)-; when -V- is -OC(O), -Z- is -NH-;

(h) -X is selected from cyclic alkyl, branched alkyl having at least two branches, and aryl, each having from 5 to about 20 carbon atoms; and

(i) -Q is selected from hydrogen; and straight or branched alkyl, saturated or unsaturated with 1 or 2 double bonds, having from 1 to about 6 carbon atoms; or -Q and -X are covalently linked forming a cyclic moiety which includes the nitrogen to which -Q is bonded and from 5 to about 20 carbon atoms.

The subject invention also involves pharmaceutical compositions comprising the above compounds, and methods for treating inflammation or pain using such compounds and compositions.

DETAILED DESCRIPTION OF THE INVENTION

The term "alkyl", as used herein, unless otherwise indicated, means carbon-containing chains which may be straight, branched or cyclic; which may be saturated or unsaturated; and which may be unsubstituted or substituted. As used herein, "cyclic alkyl" may have all or only a portion of the total number of carbon atoms indicated as being in the alkyl group in the cyclic ring itself. Cyclic alkyl includes monocycloalkyl, bicycloalkyl and/or tricycloalkyl.

Preferred alkyl are as noted in this paragraph unless provided otherwise in particular instances. Preferred alkyl are saturated. Preferred alkyl are unsubstituted. Preferred alkyl substituents are selected from halo, amino, hydroxy, alkoxy, cyano, nitro, aryl and trifluoromethyl. As used herein, "alkoxy" is -O-alkyl. It is preferred that substituted alkyl be mono-, di- or trisubstituted, especially monosubstituted.

The term "aryl", as used herein, unless otherwise specified, means aryl rings which may be unsubstituted or substituted. Preferred aryl is as noted in this paragraph, unless provided otherwise in particular instances. Preferred aryl are phenyl and naphthyl, especially phenyl. Preferred aryl are mono-, di-, tri- or unsubstituted; more preferred aryl are monosubstituted or unsubstituted, especially unsubstituted. Preferred aryl substituents include alkyl, halo, amino, hydroxy, alkoxy, cyano, nitro and trifluoromethyl.

The term "arylalkyl", as used herein, means alkyl substituted with aryl. A preferred arylalkyl is arylmethyl.

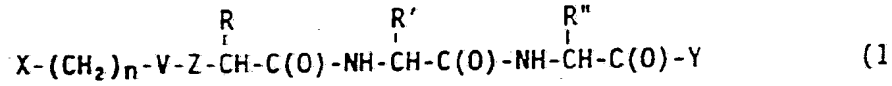
The following abbreviations are used herein. Abbreviations for amino acids may refer to the amino acid itself, or more often to an amino acid moiety that is part of a peptide or peptide derivative structure.

Amino Acid	AA
Protecting Group	PG
Arginyl	Arg
Cyclic Arginyl	cArg
Leucyl	Leu
Isoleucyl	Ile

Lysyl	Lys	
Phenylalanyl	Phe	
p-Guanidinophenylalanyl	p-Gphe	
Prolyl	Pro	
Seryl	Ser	
Threonyl	Thr	
allo-Threonyl	aThr	
Tryptophyl	Trp	
Valyl	Val	
1-Naphthylalanyl	1-Nal	collectively
2-Naphthylalanyl	2-Nal	Nal
Cyclohexylalanyl	Cha	
t-Butylalanyl	t-Bal	
1-Adamantylalanyl	1-Adl	collectively
2-Adamantylalanyl	2-Adl	Adl
Ornithyl	Orn	
Adamantylglycyl	Adg	
t-Butylglycyl	t-Bug	
Aminocyclohexyl	Ach	
Aminocyclopentyl	Acp	
Cyclohexylglycyl	Chg	
Homophenylalanyl	Hph	
p-Chlorophenylalanyl	p-Cph	
p-Nitrophenylalanyl	p-Nph	
Thienylalanyl	Thi	
Carbobenzyloxy	Cbz or Z	
t-Butyloxycarbonyl	Boc	
Adamantyloxycarbonyl	Adoc	
Benzyl	Bn	
Adamantoyl	Ad	
Adamantaneacetyl	Ada	
Menthyloxycarbonyl	Moc	
Homoadamantyloxycarbonyl	Hadoc	
Fenchyloxycarbonyl	Foc	
Isomenthyloxycarbonyl	Imoc	
Isopinocamphanyloxycarbonyl	Ipoc	
endo-Bornyloxycarbonyl	eBroc	

Naphthyloxycarbonyl	Noc
3,5-Dimethyladamantyloxycarbonyl	3,5-Dmadoc
Morphilinoyl	Mnc
Fluorenylmethoxycarbonyl	Fmoc
Noradamantoyl	Norad
Adamantylaminocarbonyl	Adac
Triphenylmethyl	Tr
Isopropylalcohol	ipa
Diethyl cyanophosphonate	DECP
Trifluoroacetate	TFA
Potassium hexamethyldisilazide	KHMDS
Lithium aluminum hydride	LAH
p-Toluenesulfonic acid	pTSA
Tetrahydrofuran	THF
Dimethylsulfoxide	DMSO
Acetyl	Ac
Methyl	Me
Ethyl	Et
3-Chloroperoxybenzoic acid	MCPBA
Dimethylformamide	DMF
Triethylamine	TEA
Diisobutylaluminum hydride	DIBAL-H
Trifluoromethyltrimethyl silane	CF_3TMS
Tetra-n-butylammonium fluoride trihydrate	TBAF
Fast atom bombardment - mass spectroscopy	FAB-MS

The novel anti-inflammatory compounds of the subject invention are those having the following chemical structure:



$$X\text{-}(CH_2)_n\text{-}V\text{-}Z\text{-}\underset{R}{CH}\text{-}C(O)\text{-}NH\text{-}\underset{R'}{CH}\text{-}C(O)\text{-}NH\text{-}\underset{R''}{CH}\text{-}C(O)\text{-}Y \qquad (1)$$

In Structure (1), n is an integer of from 0 to about 2; n is preferably 0 or 1.

In Structure (1), -R is selected from the group consisting of straight or branched alkyl, saturated or unsaturated with 1 or 2 double bonds, having from 1 to about 6 carbon atoms; and cyclic alkyl, saturated or unsaturated with 1 or 2 double bonds, having

from 3 to about 13 carbon atoms. Preferred -R is saturated alkyl. Preferred alkyl are unsubstituted, or are arylalkyl, especially benzyl and naphthal. Preferred cyclic alkyl are cyclic C_3-C_8 (more preferably C_5-C_6) methyl or adamantylmethyl. Preferred -R is hydrophobic, preferably with the hydrophobicity concentrated close to the carbon atom to which -R is bonded. Specific examples of preferred -R include t-butyl, 1,1-dimethyl-propyl, i-propyl, i-butyl, s-butyl, neo-pentyl, cyclohexyl, cyclohexylmethyl, adamantyl, naphthal; most preferred -R is t-butyl.

In Structure (1), the carbon to which -R is bonded is in either D or L, preferably D, configuration. The structure -Z-CH(R)-C(O)- is an amino acid moiety (hereinafter -AA^1-) when -Z- is -NH; preferred amino acid moieties for -AA^1- include t-Bug, Val, Ile, Leu, Chg, Cha, Phe, Nal, Trp and Adg; more preferred are t-Bug, Val and Ile; most preferred -AA^1- is t-Bug.

In Structure (1), -R′ is selected from branched alkyl, saturated or unsaturated with 1 or 2 double bonds, having from 3 to about 6 carbon atoms; cyclic alkyl, saturated or unsaturated with 1 or 2 double bonds, having from 3 to about 13 carbon atoms; and arylalkyl, the alkyl portion being saturated and having from 1 to about 3 carbon atoms. Preferred branched or cyclic alkyl are saturated. Preferred branched or cyclic alkyl are unsubstituted. Preferred branched alkyl have from 3 to 5 carbon atoms; most preferred branched alkyl is i-butyl. Preferred cyclic alkyl have a C_3-C_7, more preferably C_5-C_6, cyclic ring bonded to a methylene, ethylene or n-propylene (preferably methylene or ethylene, more preferably methylene) which is bonded to the carbon atom in Structure (1) to which -R′ is bonded. Preferred arylalkyl are benzyl, p-hydroxybenzyl and naphthal. More preferred -R′ is selected from i-propyl, i-butyl, s-butyl, cyclohexylmethyl, benzyl and naphthal; most preferred is benzyl.

In Structure (1), the carbon atom to which -R′ is bonded is in L configuration. The structure -NH-CH(R′)-C(O)- is an amino acid moiety (hereinafter -AA^2-); preferred amino acid moieties for -AA^2- include Phe, Nal, Cha, Leu and Ile; most preferred -AA^2- is Phe.

In Structure (1), -R" is $-(CH_2)_m$-A-NH_2 or $-(CH_2)_m$-A-B-$C(NH_2)$=NH, wherein m is an integer of from 1 to about 5, -A- is a covalent bond, p-phenyl or p-cyclohexyl, and -B- is a covalent bond or -NH-. When -A- is a covalent bond, m is preferably 2-5, more preferably 2-4, more preferably still 3 or 4. When -A- is p-phenyl or p-cyclohexyl, m is preferably 1-4, more preferably 1-3, more preferably still 1. -A- is preferably a covalent bond. -B- is preferably -NH-. More preferred -R" is 3-guanidino-n-propyl or 4-amino-n-butyl.

In Structure (1), the carbon to which -R" is bonded is in L configuration. The structure -NH-(CH(R")-C(O)- is an amino acid moiety (hereinafter $-AA^3$-); preferred amino acid moieties for $-AA^3$- include Arg, Lys and p-Gphe; most preferred $-AA^3$- is Arg.

In Structure (1), -Y is hydrogen or trifluoromethyl.

In Structure (1), -Z- is -O- or -NH-. Preferred -Z- is -NH-.

In Structure (1), -V- is selected from -OC(O)-, -N(Q)C(O)-, -N(Q)C(S)-, -C(O)-, $-SO_2$- and -P(O)(OH)-. Preferred -V- is selected from -OC(O)-, -N(Q)C(O)-, -N(Q)C(S)-, and -C(O)-. More preferred -V- is -OC(O)- or -N(Q)C(O)-. Most preferred -V- is -OC(O)-. When -V- is -OC(O)-, -Z- is -NH-.

In Structure (1), -X is selected from cyclic alkyl, branched alkyl having at least 2 branches, and aryl, each having from 5 to about 20 carbon atoms. Preferred -X has from 5 to 15 carbon atoms; more preferred -X has from 8 to 12 carbon atoms. Preferred alkyl portions of -X are saturated. Preferred -X is unsubstituted or substituted with unsubstituted alkyl or aryl. Preferred cyclic alkyl are monocycloalkyl, bicycloalkyl, and tricycloalkyl, more preferred are bicycloalkyl and tricycloalkyl, especially tricycloalkyl. Preferred cycloalkyl have 5 or 6 carbon atoms, more preferably 6 carbon atoms, in each cyclic ring. A highly preferred -X is adamantyl. Preferred aryl -X are naphthyl and phenyl, unsubstituted or substituted with alkyl. Particularly preferred aryl -X include naphthyl and fluorenyl. When -X is aryl or cyclic alkyl, n is preferably 1.

In Structure (1) when -V- is -N(Q)C(O)- or -N(Q)C(S)-, -Q is selected from hydrogen; straight or branched alkyl, saturated or

unsaturated with 1 or 2 double bonds, having from 1 to about 6 carbon atoms; or -Q and -X are covalently linked forming a cyclic moiety which includes the nitrogen to which -Q is bonded and from 5 to about 20 carbon atoms. Preferred -Q-X- has from 5 to 15 carbon atoms; more preferred -Q-X- has from 8 to 12 carbon atoms. Preferred -Q-X- is unsubstituted or substituted with unsubstituted alkyl or aryl. Preferred cyclic

is monocyclic, bicyclic or tricyclic; more preferred is monocyclic. Most preferred -Q is hydrogen.

In Structure (1), preferred $X-(CH_2)_n-V-$ (hereinafter X'-) include Boc, Adoc, Ad, Fmoc, Ada, Adac, Mnoc, Norad, Hadoc, Foc, Imoc, Ipoc, 3,5-Dmadoc, eBroc, Noc and Moc; more preferred X' is Adoc, Ipoc and eBroc; most preferred -X' is Adoc.

Preferred anti-inflammatory compounds of the subject invention include pharmaceutically-acceptable salts of the above compounds. Particularly preferred salts include salts of addition formed between a strong acid and the above compounds. Particularly preferred are such salts of addition where -R" is protonated, resulting in a positive charge on the -R" moiety. Preferred compounds of the subject invention are often associated with one or more molecules of water in the form of hydrates.

Preferred anti-inflammatory compounds of the subject invention include the following compounds:

Boc-D-Phe-Phe-Arg-H
Boc-D-1-Nal-Phe-Arg-H
Boc-D-Phe-Cha-Arg-H
Boc-D-Cha-Cha-Arg-H
Boc-D-Phe-Thi-Arg-H
Boc-D-Cha-Phe-Arg-H
Boc-D-Hph-Phe-Arg-H
Adoc-D-Phe-Phe-Arg-H
Boc-D-Phe-Hph-Arg-H
Boc-D-Phe-2-Nal-Arg-H
Boc-D-2-Nal-Phe-Arg-H
Adoc-D-2-Nal-Phe-Arg-H
Adoc-D-2-Nal-2-Nal-Arg-H

Ad-D-Phe-Phe-Arg-H
Boc-D-Trp-Phe-Arg-H
Boc-D-t-Bug-Phe-Arg-H
Fmoc-Val-Phe-Arg-H
Adoc-D-Hph-Phe-Arg-H
Fmoc-D-Phe-Phe-Arg-H
Boc-D-t-Bal-Phe-Arg-H
Boc-D-1-Adl-Phe-Arg-H
Boc-D-2-Adl-Phe-Arg-H
Adoc-D-t-Bug-Phe-Arg-H
Ad-D-Phe-Phe-Arg-CF_3
Adoc-D-Val-Phe-Arg-H
Adoc-D-t-Bug-2-Nal-Arg-H
Ada-D-t-Bug-Phe-Arg-H
Adoc-D-Chg-Phe-Arg-H
Adoc-D-t-Bug-p-Cph-Arg-H
Adoc-D-t-Bug-Trp-Arg-H
Adoc-L-Val-Phe-Arg-H
Adoc-Ach-Phe-Arg-H
Adoc-Ach-Phe-Arg-H
Adoc-D-Adg-Phe-Arg-H
Adoc-D-Orn-Phe-Arg-H
Adoc-D-aThr-Phe-Arg-H
Adoc-D-Leu-Phe-Arg-H
Adoc-D-Lys-Phe-Arg-H
Adac-D-t-Bug-Phe-Arg-H
(-)-Moc-D-t-Bug-Phe-Arg-H
Norad-D-t-Bug-Phe-Arg-H
Norad-D-t-Bug-Phe-Arg-H
Adoc-D-t-Bug-Phe-Lys-H
Hadoc-D-t-Bug-Phe-Arg-H
Adoc-D-t-Bug-Cha-Arg-H
Adoc-D-t-Bug-Leu-Arg-H
Foc-D-t-Bug-Phe-Arg-H
Imoc-D-t-Bug-Phe-Arg-H
(-)-Ipoc-D-t-Bug-Phe-Arg-H
3,5-Dmadoc-D-t-Bug-Phe-Arg-H

(+)-Ipoc-D-t-Bug-Phe-Arg-H
(-)-eBroc-D-t-Bug-Phe-Arg-H
Adoc-D-t-Bug-Phe-Arg-H
Noc-D-t-Bug-Phe-Arg-H
Adoc-D-t-Bug-Phe-Arg-CF_3
Adoc-D-t-Bug-Phe-p-Gphe-CF_3
eBroc-D-t-Bug-Phe-Arg-CF_3·TFA salt
Boc-D-Cha-Cha-p-Gphe-CF_3
Adoc-D-Val-Phe-p-Gphe-CF_3
Boc-D-Phe-2-Nal-p-Gphe-CF_3
Adac-D-t-Bug-Phe-Arg-CF_3
Mnc-D-Phe-2-Nal-Arg-CF_3

Amino acids are in L configuration, unless indicated as D in the above list.

Pharmaceutical Compositions

Pharmaceutical compositions of the subject invention comprise a safe and effective amount of a tripeptide derivative as defined hereinabove and a pharmaceutically-acceptable carrier. Such compositions typically comprise from about 0.1% to about 95% by weight of the tripeptide derivative, preferably from about 1% to about 90% and more preferably from about 5% to about 75%.

The term "pharmaceutically-acceptable carrier", as used herein, means one or more compatible solid or liquid filler diluents or encapsulating substances which are suitable for administration to a human or lower animal. The term "compatible", as used herein, means that the components of the pharmaceutical compositions are capable of being commingled with the tripeptide derivative of the subject invention, and with each other, in a manner such that there is no interaction which would substantially reduce the pharmaceutical efficacy of the composition under ordinary use situations. Pharmaceutically-acceptable carriers must, of course, be of sufficiently high purity and sufficiently low toxicity to render them suitable for administration to the human or lower animal being treated.

Some examples of substances which can serve as pharmaceutically-acceptable carriers are sugars, such as lactose, glucose and sucrose; starches, such as cornstarch and potato

starch; cellulose and its derivatives, such as sodium carboxymethylcellulose, ethylcellulose and cellulose acetate; powdered tragacanth; malt; gelatins; talc; stearic acid; magnesium stearate; calcium sulfate; vegetable oils, such as peanut oil, cottonseed oil, sesame oil, olive oil, corn oil and oil of theobroma; polyols, such as propylene glycol, glycerin, sorbitol, mannitol, and polyethylene glycol; alginic acid; pyrogen-free water; isotonic saline; phosphate buffer solutions; cocoa butter (suppository base); emulsifiers, such as Tweens®; wetting agents and lubricants, such as sodium lauryl sulfate; coloring agents; flavoring agents; stabilizers; antioxidants; preservatives; as well as other non-toxic compatible substances used in pharmaceutical formulations. Other compatible pharmaceutical additives and actives may be included in the pharmaceutically-acceptable carrier for use in the compositions of the subject invention.

The choice of a pharmaceutically-acceptable carrier to be used in conjunction with the tripeptide derivatives of the subject invention is determined by the way the active is to be administered. Preferred modes of administering the actives of the subject invention are by injection, ingestion, inhalation and topically.

The pharmaceutically-acceptable carrier employed in conjunction with the tripeptide derivative of the subject invention is used at a concentration sufficient to provide a practical size to dosage relationship. The pharmaceutically-acceptable carrier, in total, typically comprises from about 5% to about 99.9% by weight of the pharmaceutical compositions of the subject invention, preferably from about 10% to about 99%, and more preferably from about 25% to about 95%.

Total single dosages of the tripeptide derivatives of the subject invention in pharmaceutical compositions are generally from about 1 mg to about 1000 mg, preferably from about 50 mg to about 800 mg, more preferably from about 100 mg to about 500 mg.

Methods for Producing Anti-inflammatory Activity and Analgesia

The subject invention also encompasses methods of producing anti-inflammatory activity and/or analgesia in humans or lower

animals through administering, to the human or lower animal in need of such treatment, a safe and effective amount of a tripeptide derivative of the subject invention. The amount can be given in a single dose or multiple doses repeatedly over the course of the treatment. While dosages higher than those described herein are effective to reduce inflammation and produce analgesia, care must be taken in some individuals to prevent adverse side effects. The tripeptide derivatives and compositions of the subject invention can be used to reduce inflammation in various disorders at the deeper structures, muscles, tendons, bursa and joints associated with disease and trauma, to treat and prevent pain.

The preferred modes of administering the tripeptide derivatives of the subject invention are by injection, ingestion, inhalation and topically. Specific modes of administration include, without limitation, oral ingestion; injection, such as intramuscular, intravenous, intraperitoneal, intradermal and subcutaneous; inhalation; and topically, such as transdermally, orally, mucosally and sublingually.

The term "safe and effective amount", as used herein, means an amount of a tripeptide derivative or composition high enough to significantly positively modify the condition to be treated, but low enough to avoid serious side effects (at a reasonable benefit/risk ratio), within the scope of sound medical judgement. A safe and effective amount of a tripeptide derivative or composition will vary with the particular condition being treated, the age and physical condition of the patient being treated, the severity of the condition, the duration of the treatment, the nature of concurrent therapy, the specific active employed, the particular pharmaceutically-acceptable carrier utilized, and like factors within the knowledge and expertise of the attending physician.

Preferred daily dosages of the tripeptide derivatives of the subject invention range from about 0.1 mg/kg of body weight to about 500 mg/kg of body weight, more preferably from about 1 mg/kg to about 100 mg/kg, still more preferably from about 2 mg/kg to about 50 mg/kg. From 1 to about 4 single dosages per

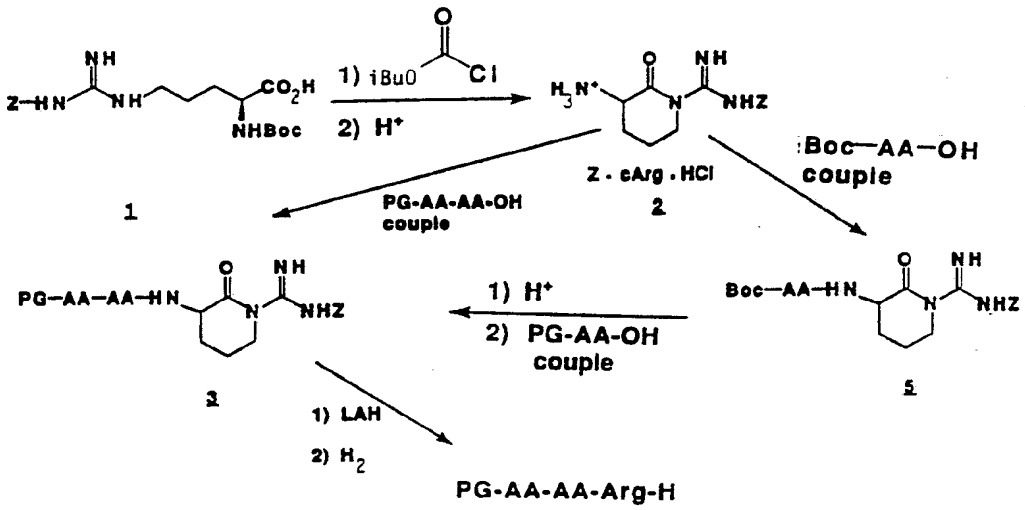
day may be administered, more preferably from about 2 to about 3 single dosages per day. Preferred amounts of the tripeptide derivatives administered by injection are from about 0.1 mg/kg/day to about 50 mg/kg/day, more preferably from about 1 mg/kg/day to about 10 mg/kg/day. Preferred amounts of the tripeptide derivatives administered by oral ingestion are from about 1 mg/kg/day to about 500 mg/kg/day, more preferably from about 5 mg/kg/day to about 100 mg/kg/day. Preferred amounts of the tripeptide derivatives administered by inhalation are from about 0.1 mg/kg/day to about 500 mg/kg/day, more preferably from about 5 mg/kg/day to about 100 mg/kg/day. Preferred amounts of the tripeptide derivatives administered topically are from about 1 mg/kg/day to about 500 mg/kg/day, more preferably from about 50 mg/kg/day to about 250 mg/kg/day.

Methods for Synthesizing the Compounds

The following non-limiting schemes and examples demonstrate methods of synthesizing the tripeptide derivatives of the subject invention.

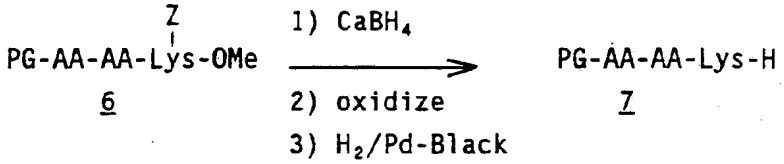
Peptide aldehydes are synthesized according to Schemes A and B hereinbelow. In Scheme A, the Z-protected arginine derivative 1 is cyclized utilizing isobutyl chloroformate and an appropriate base in an inert solvent and deblocked with anhydrous HCl to give the cyclic arginine derivative 2: (Z)-cArg·HCl. This compound can be coupled with a dipeptide unit with a protecting group on the amino terminus utilizing diethyl cyanophosphonate and an appropriate amine base at room temperature to form the tripeptide nucleus 3. The ester 3 can be effectively reduced with lithium aluminum hydride in THF at -20°C and the protecting group (Z) removed by catalytic hydrogenolysis to yield the target peptide 4. In a similar sequence of reactions, the cyclic arginine derivative 2 is coupled to a Boc-protected amino acid under conditions identical to those utilized for the formation of 3 to form a dipeptide unit 5. The Boc group is then removed with anhydrous HCl in dioxane and the resulting amine salt coupled with another amino acid derivative under the same conditions after exposure to base. This again forms the tripeptide nucleus 3. As before this compound is converted to 4.

Scheme A



In scheme B, the tripeptide ester 6 which is synthesized utilizing standard peptide chemistry is reduced to the alcohol with $CaBH_4$ at 0°C in THF and subsequently oxidized with DMSO and oxalyl chloride to give the protected peptide aldehyde. Removal of the protecting group by catalytic hydrogenolysis affords the target peptide aldehyde.

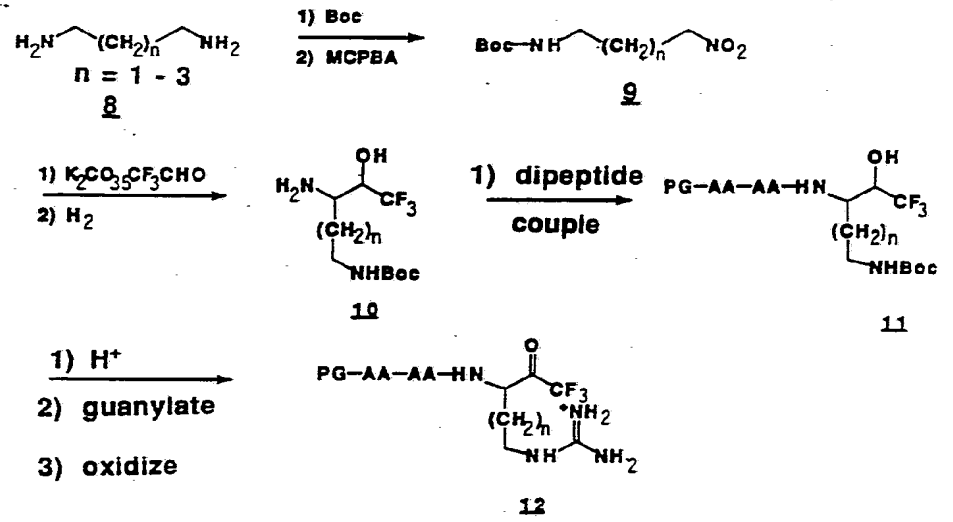
Scheme B



Trifluoromethyl ketones are synthesized according to Schemes C and D hereinafter. In Scheme C, a diamine 8 is monoprotected as the Boc derivative and the resulting free amine oxidized with warm 3-chloroperoxybenzoic acid in dichloroethylene to give the nitro derivative 9. This compound is condensed with CF_3CHO hydrate in THF to afford a nitro alcohol and the nitro reduced to the amine to give 10. Subsequent reaction of this free amine

with a dipeptide unit in the presence of diethyl cyanophosphonate and a suitable amine is followed by selective removal of the Boc with anhydrous acid, guanylation of the resulting free amine with 3,5-dimethylpyrazole-carboxamidine nitrate in dry DMF and oxidation with Dess-Martin periodinane (see Dess, D.B. & J.C. Martin, "Readily Accessible 12-I-5 Oxidant for the Conversion of Primary and Secondary Alcohols to Aldehydes and Ketones", Journal of Organic Chemistry, Vol. 48 (1983), pp. 4155-4156) to give the trifluoromethyl ketone 12.

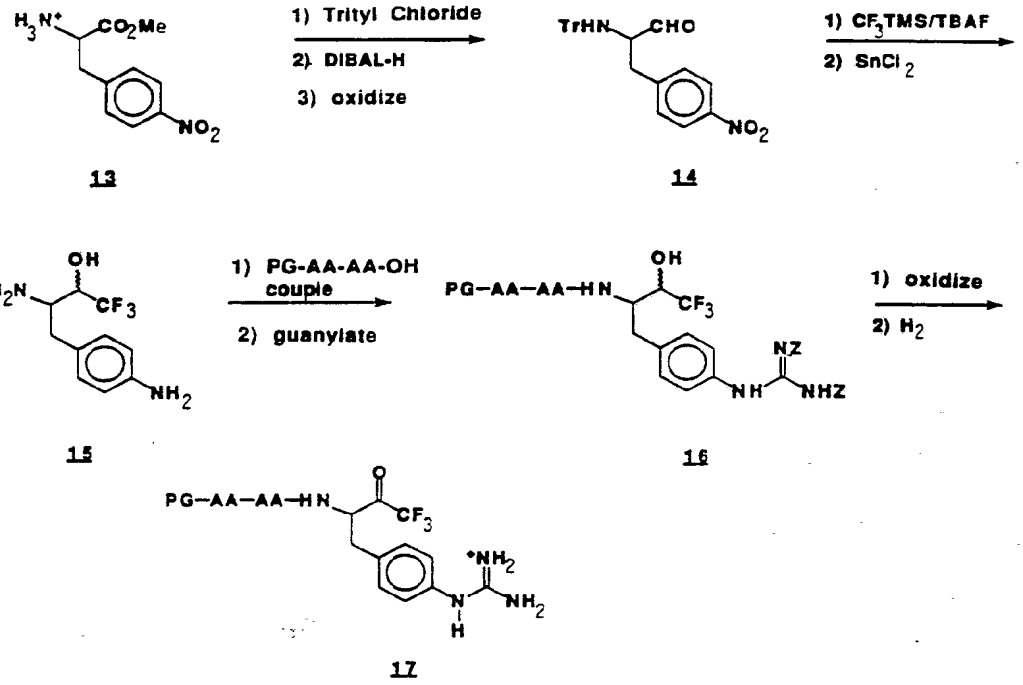
Scheme C



Other trifluoromethyl ketones are synthesized by a different route. In Scheme D, ester 13 is tritylated in dichloromethane with trityl chloride and TEA, and the ester is reduced with DIBAL-H in THF and oxidized to the aldehyde 14 under conditions identical to those utilized in the conversion of 6 to 7. This compound is reacted with CF_3TMS (see Krishnamurti, R., D.R. Bellew & G.K.S. Prakash, "Preparation of Trifluoromethyl and Other Perfluoroalkyl Compounds with (Perfluoroalkyl)trimethylsilanes", Journal of Organic Chemistry, Vol. 56 (1991),

pp. 984-989) and tetra-n-butylammonium fluoride trihydrate in THF, and the product is doubly reduced with anhydrous tin dichloride to give the diamine 15. Selective coupling of a dipeptide unit is effected at the less nucleophilic amino site with diethyl cyanophosphonate and an appropriate base, and the remaining aniline is guanylated with N,N'-bis-Cbz-S-methyl-isothiourea (Example 74) and a mercuric acetate catalyst to give 16. Oxidation of the alcohol with Dess-Martin periodinane and removal of the Z protecting groups by hydrogenolysis with palladium black in DMF gives the trifluoromethyl ketone 17.

Scheme D



13 14 15 16 17

Example 1

N-(Cbz)amidino-2-(Boc)aminovalerolactam: α-Boc,ω-Cbz-arginine (5.0 g, 9.84 mmol) is cooled to 0°C in 150 ml THF under

an inert atmosphere. To this solution is added 1.5 ml (10.82 mmol) TEA dropwise followed by the dropwise addition of 1.4 ml (10.82 mmol) of isobutyl chloroformate. After 0.5 h at 0°C, the cooling bath is removed, and the mixture is stirred at ambient temperature for 4 h. The reaction mixture is poured into a mixture of ethyl acetate (100 ml) and saturated sodium bicarbonate, and the phases are separated in a separatory funnel. The organic phase is washed with saturated sodium chloride, dried with magnesium sulfate and filtered, and the solvent is removed. The residue is chromatographed on silica gel to afford 4.0 g product. Recrystallization from acetone gives analytically pure product. R_f = 0.55 (5% ipa/CH_2Cl_2).

Example 2

Boc-D-Phe-Phe-OBn: To a slurry of Boc-D-Phe-OH (1.00 g, 3.77 mmol) and Phe-OBn·p-TSA salt (1.61 g, 3.77 mmol) in 50 ml dichloromethane under argon is added 1.27 ml (9.05 mmol) TEA followed by the addition of 0.682 ml (4.5 mmol) of diethyl cyanophosphonate (DECP). The mixture is stirred overnight and the solvent is removed. Flash chromotography on silica gel gives 2.01 g of the desired compound.

Example 3

N-(Cbz)amidino-2-aminovalerolactam·HCl salt ((Z)-cArg·HCl): To a solution of 1.35 g (3.45 mmol) N-(Cbz)amidino-2-(Boc)amino-valerolactam (Example 1) in 25 ml dioxane is added 15 ml of 3.38M HCl in dioxane. The solution is stirred at room temperature for 1.5 h, and the solvent is removed. The residue is triturated with ether and the solids filtered under argon to give 1.05 g of white crystalline product.

Example 4

Boc-D-Phe-Phe-OH: To a solution of 0.520 g (1.04 mmol) Boc-D-Phe-Phe-OBn (Example 2) in 10 ml MeOH is added 0.250 g palladium on carbon and 0.5 ml acetic acid. The slurry is hydrogenated on the Paar® apparatus at 45-50 psi overnight. The catalyst is filtered through Celite®, and the solvent is removed to afford 0.204 g product.

Example 5

Boc-D-Phe-Phe-(Z)-cArg: To a solution of Boc-D-Phe-Phe-OH (Example 2) (0.390 g, 0.980 mmol) and (Z)-cArg·HCl salt (Example 3) (0.432 g, 0.980 mmol) in dichloromethane is added 0.30 ml (2.16 mmol) TEA followed by 0.17 ml (1.08 mmol) diethyl cyanophosphonate. The mixture is stirred overnight and washed with 1N HCl, saturated sodium bicarbonate, and saturated sodium chloride. Flash chromotography on silica gel gives 0.400 g product. R_f= 0.45 (5% ipa/CH_2Cl_2).

Example 6

Boc-D-Phe-Phe-Arg-H·OAc: To a cold (-23°C) solution of Boc-D-Phe-Phe-(Z)-cArg (Example 5) (0.170 g, 0.250 mmol) in 15 ml THF is added 0.019 g (0.500 mmol) of LAH portionwise over a period of 1 min. After stirring for 1 h at this temperature the reaction is quenched with 1 ml of water, warmed to room temperature and filtered, and the solvent is removed. The residue is dissolved in dichloromethane and washed with brine. The resulting organic phase is dried with magnesium sulfate and filtered, and the solvent is removed. The resulting alcohol is dissolved in 10 ml methanol, and 0.100 g palladium on carbon is added. Hydrogen is bubbled through the slurry for 1 h, and the slurry is filtered through Celite®; the solvent is removed. The residue is dissolved in 7% acetic acid/water and lyophilized to afford 0.85 g product. FAB-MS 553 m/z (MH^+), 575 m/z $(MNa)^+$, 535 m/z $(MH-H_2O)^+$.

Example 7

Boc-D-Pro-Phe-(Z)-cArg: To a solution of Boc-D-Pro-Phe-OH (0.357 g, 0.810 mmol) and (Z)-cArg·HCl in 25 ml dichloromethane is added 0.250 ml (1.78 mmol) TEA dropwise followed by 0.140 ml (0.890 mmol). The solution is stirred overnight, and the solvent is removed. Chromotography on silica gel gives 0.362 g product. R_f = 0.40 (5% ipa/CH_2Cl_2).

Example 8

Boc-D-Pro-Phe-Arg-H·OAc: As in Example 6, 0.285 g (0.460 mmol) of Boc-D-Pro-Phe-(Z)-cArg (Example 7) and 0.035 g (0.92 mmol) LAH gives crude product. This product is chromatographed on silica gel to give 0.100 g product, which is then hydrogenated

(0.100 g palladium on carbon) to afford 0.088 g of the desired product.

Example 9

Boc-D-1-Nal-Phe-OBn: As in Example 2, 0.177 g Boc-D-1-Nal-OH (0.560 mmol) and 0.265g Phe-OBn·p-TSA (0.620 mmol) with 0.100 ml DECP (0.620 mmol) and 0.160 ml (1.12 mmol) gives 0.230 g product. R_f= 0.75 (3% ipa/CH_2Cl_2).

Example 10

Boc-D-1-Nal-Phe-(Z)-cArg: As in Example 5, 0.130 g (0.270 mmol) Boc-D-1-Nal-Phe-OH (see Example 4 for preparation method used to make an analogous compound) and 0.122 g (Z)-cArg·HCl (0.270 mmol) with 0.076 ml TEA (0.540 mmol) and 0.051 ml DECP (0.300 mmol) gives 0.135 g product. R_f = 0.50 (5% ipa/CH_2Cl_2).

Example 11

Boc-D-1-Nal-Phe-Arg-H·OAc: As in Example 6, 0.130 g (0.180 mmol) Boc-D-1-Nal-Phe-(Z)-cArg (Example 10) and 0.014 g LAH (0.360 mmol) gives 0.078 g chromatographed product that is hydrogenated and lyophilized to give 0.049 g product. FAB-MS 603 m/z (MH^+).

Example 12

Boc-D-Phe-Cha-OBn: As in Example 2, 0.223 g (0.840 mmol) Boc-D-Phe-OH and 0.250 g Cha-OBn·HCl (Example 76) (0.840 mmol) with 0.140 ml (0.923 mmol) DECP and 0.234 ml (1.68 mmol) TEA gives 0.262 g product. R_f = 0.51 (25% EtOAc/ pentane).

Example 13

Boc-D-Phe-Cha-OH: As in Example 4, 0.262 g (0.515 mmol) Boc-D-Phe-Cha-OBn (Example 12) and 0.110 g palladium on carbon gives 0.212 g product.

Example 14

Boc-D-Phe-Cha-(Z)-cArg: As in Example 5, 0.165 g (0.507 mmol) (Z)-cArg·HCl (Example 3) and 0.212 g (0.507 mmol) Boc-D-Phe-Cha-OH (Example 13) with 0.085 ml (0.557 mmol) DECP and 0.141 ml (1.01 mmol) TEA gives 0.051 g product. R_f= 0.62 (5% ipa/CH_2Cl_2).

Example 15

Boc-D-Phe-Cha-Arg-H·OAc: As in Example 6, 0.049 g (0.071 mmol) Boc-D-Phe-Cha-(Z)-cArg (Example 14) and 0.005 g LAH gives 0.020 g chromatographed product that is hydrogenated (0.007 g Pd/C) and lyophilized to afford 0.008 g product. FAB-MS 559 m/z (MH^+).

Example 16

Adoc-D-Phe-Phe-OBn: Boc-D-Phe-Phe-OBn (Example 2) (0.500 g, 0.995 mmol) is dissolved in 5 ml of ethyl acetate, and 5ml of 5M HCl is added. After 2.5 h the solvent is removed, and the residue is placed on the vacuum pump overnight. The solid obtained (D-Phe-Phe-OBn·HCl) is dissolved in a mixture of 1 ml of dioxane and 0.995 ml of 1N NaOH. The solution is cooled to 0°C and 0.092 g (1.09 mmol) sodium bicarbonate is added. To this solution is added 0.092 g (1.094 mmol) adamantyl fluoroformate in 1 ml dioxane dropwise via syringe. The solution is stirred at 0°C for 0.5 h and for 1.5 h at room temperature. The solvent is evaporated, and the residue is partitioned between ethyl acetate and water. The phases are separated, and the aqueous phase is extracted with 3 X 20 ml ethyl acetate. The combined organic phases are washed with brine, dried with magnesium sulfate, and filtered, and the solvent is removed. The product is chromatographed on silica gel to give 0.390 g of the desired product. R_f = 0.70 (5% ipa/CH_2Cl_2).

Example 17

Adoc-D-Phe-Phe-OH: As in Example 4, 0.380 g (0.653 mmol) of Adoc-D-Phe-Phe-OBn (Example 16) and 0.076 g Pd/C gives 0.192 g of the desired product.

Example 18

Adoc-D-Phe-Phe-(Z)-cArg: As in Example 5, 0.100 g (0.204 mmol) of Adoc-D-Phe-Phe-OH (Example 17) and 0.061 g (0.185 mmol) of (Z)-cArg·HCl (Example 3) with 0.031 ml (0.204) DECP and 0.057 ml (0.408 mmol) TEA gives 0.079 g product. R_f= 0.53 (5% ipa/CH_2Cl_2).

Example 19

Adoc-D-Phe-Phe-Arg-H·OAc: As in Example 6, 0.078 g (0.102 mmol) Adoc-D-Phe-Phe-(Z)-cArg (Example 18) and 0.008 g

(0.204 mmol) LAH gives 0.064 g product that is hydrogenated and lyophilized to afford 0.059 g product. FAB-MS 631 m/z (MH^+).

Example 20

Adoc-D-2-Nal-Phe-(Z)-cArg: To 0.046 g (0.062 mmol) of Boc-D-2-Nal-Phe-(Z)-cArg (see Example 10 for preparation method used to make an analogous compound) in 5 ml ethyl acetate is added 1 ml of 5M HCl in ethyl acetate. The solution is stirred for 8 h; the solvent is removed, and the residue is placed on the vacuum pump for several hours. The resulting amine salt (D-2-Nal-Phe-(Z)-cArg·HCl) is dissolved in 0.5 ml of dioxane and 0.5 ml of water. 0.045 ml of 1N NaOH is added followed by 0.004 g (0.049 mmol) of sodium bicarbonate. 0.010 g (0.049 mmol) of adamantyl fluoroformate in 1 ml dioxane is added dropwise, and the solution is stirred at 0°C for 0.5 h and at room temperature for 2 h. The solvent is evaporated, and the residue is partitioned between ethyl acetate and water. The phases are separated, and the aqueous phase is extracted with ethyl acetate. The organic phases are combined and washed with brine, dried with magnesium sulfate and filtered, and the solvent is removed. Chromatography on silica gel gives 0.021 g product. R_f = 0.41 (5% ipa/CH_2Cl_2).

Example 21

Adoc-D-2-Nal-Phe-Arg-H·OAc: As in Example 6, 0.009 g (0.011 mmol) Adoc-D-2-Nal-Phe-(Z)-cArg (Example 20) and 0.850 mg of LAH gives 0.007 g product that is hydrogenated (0.002 g Pd/C) and lyophilized to afford 0.004 g of the desired product. FAB-MS 681 m/z (MH^+).

Example 22

Boc-Phe-(Z)-cArg: To a solution of Boc-Phe-OH (1.30 g, 4.90 mmol) and (Z)-cArg·HCl (Example 3) (1.60 g, 4.90 mmol) in 25 ml dichloromethane under an inert atmosphere is added in succession 1.43 ml (10.3 mmol) TEA and 0.743 ml (4.90 mmol) DECP. After stirring overnight the solvent is removed, and the residue is chromatographed on silica gel to afford 1.44 g product.

Example 23

Phe-(Z)-cArg·HCl: Boc-Phe-(Z)-cArg (Example 22) (1.44 g, 2.68 mmol) is dissolved in 20 ml ethyl acetate and 22 ml of a 3M

solution of HCl in ethyl acetate is added. The solution is stirred for 4 h, and the resulting solid material is filtered through a Buchner funnel with a continuous stream of argon passing over any exposed solid material. The filter cake is washed with ethyl acetate and placed under vacuum for future use. 1.23 g of white solid is obtained.

Example 24

Boc-D-Trp-Phe-(Z)-cArg: To a solution of 0.150 g (0.320 mmol) Phe-(Z)-cArg·HCl (Example 23) and 0.097 g (0.320 mmol) Boc-D-Trp-OH in 25 ml dichloromethane under an inert atmosphere is added 0.090 ml TEA (0.640 mmol), followed by the addition of 0.060 ml DECP (0.360 mmol); the resulting solution is stirred overnight. The solvent is removed, and the residue is chromatographed on silica gel to give 0.160 g product. R_f = 0.40 (5% ipa/CH_2Cl_2).

Example 25

Boc-D-Trp-Phe-Arg-H·OAc: As in Example 6, 0.110 g (0.150 mmol) Boc-D-Trp-Phe-(Z)-cArg (Example 24) and 0.011 g LAH gives 0.065 g chromatographed product that is hydrogenated (0.050 g Pd/C) and lyophilized to afford 0.040 g product. FAB-MS 592 m/z (MH^+), 700 m/z (MH + TG)$^+$, 492 m/z (MH-Boc)$^+$.

Example 26

N-Bn-D-Ser-OH: 10.0 g (95.2 mmol) of D-Serine and 4.78 g (76.12 mmol) of sodium cyanoborohydride are suspended in 200 ml methanol, and 10.6 g (104 mmol) benzaldehyde is added dropwise. The slurry is stirred for 48 h and filtered. The filter cake is washed with methanol and placed on the vacuum pump overnight. 10.5 g of a white powder that is a 1:1 mixture of starting material and product is obtained.

Example 27

N-Bn,N-(Z)-D-Ser: A 1:1 mixture of N-Bn-D-Ser-OH and D-Serine (Example 26) (5.00 g) is dissolved in 18.3 ml 2N NaOH and 6 ml THF is added. The solution is cooled to 0°C, and 7.86 ml (55.0 mmol) benzylchloroformate is added. During addition the pH is maintained between 9.5 and 10.5 by addition of 1N NaOH. After 1 h of stirring and continuously maintaining the pH, the solution is acidified to pH = 2.4 with concentrated HCl. The

acidic solution is extracted with ethyl acetate (3X) and dried with magnesium sulfate; and the solvent is removed. The residue is chromatographed on silica gel to afford 4.00 g of the desired product. R_f = 0.27 (1% acetic acid/5% ipa/94% dichloromethane).

Example 28

N-Bn-N-(Z)-D-Ser-β-lactone: To a dry flask under argon containing 1.52 g triphenylphosphine (5.77 mmol) in 20 ml THF at -78°C is added 0.637 ml dimethylazodicarboxylate (5.79 mmol) dropwise. After 15 min, a solution of N-Bn-N-(Z)-D-Ser (Example 27) in 15 ml THF is added. The cooling bath is removed, and the solution is allowed to warm to room temperature overnight. The solution is evaporated, and the residue is chromatographed on silica gel to give 1.00 g product. R_f = 0.37 (20% ethyl acetate/petroleum ether).

Example 29

N-Bn-N-(Z)-D-tBal-OH: To a slurry of cuprous bromide/dimethylsulfide complex (0.581 g, 2.83 mmol) in 5 ml THF in a previously dried flask at -78°C under argon is added 2.96 ml t-BuLi (1.7M in pentane, 5.04 mmol) dropwise. The slurry is stirred at this temperature for 0.75 h and 0.3 h at -45°C. The slurry is recooled to -78°C, and 0.200 g (0.642 mmol) of N-Bn-N-(Z)-D-Ser-β-lactone (Example 28) in 2 ml THF is added. After stirring for 16 h at -78°C, the mixture is poured into cold 1N HCl, and methanol is added (25% volume). After 0.3 h the mixture is filtered, and the filtrate is extracted with ethyl acetate and dried with magnesium sulfate; the solvent is removed. Chromatography of the residue on silica gel gives 0.120 g product. $[\alpha]_D^{25}$ = +32.18 (c = 1, chloroform). R_f = (1% HOAc/5% MeOH/94% CH_2Cl_2).

Example 30

Boc-D-t-Bal-OH: To a solution of N-Bn-N-(Z)-D-tBal-OH (Example 29) (0.160 g, 0.432 mmol) in water:acetic acid (1:2, 15 ml) is added 0.030 g Pd/C and the slurry hydrogenated on the Paar® apparatus at 45 psi for 16 h. After filtering through Celite®, the solvent is removed, and the residue is placed on the vacuum pump. The deprotected product is dissolved in 0.480 ml 1N

NaOH and 2 ml dioxane, and 1 ml water is added. The solution is cooled to 0°C and 0.480 ml 1N NaOH and 0.078 g di-t-butyldicarbonate (0.465 mmol) in 1 ml dioxane are added simultaneously in separate addition funnels, and the resulting solution is stirred at 0°C for 1 h. The solution is extracted with ethyl acetate, and the aqueous phase is acidified to pH = 2.5. Extraction of the aqueous phase with ethyl acetate is followed by drying with magnesium sulfate, filtering and removal of solvent. The residue is chromatographed on silica gel to give 0.109 g of pure material. $[\alpha]_D^{25}$ = +32.7 (c = 1, chloroform). R_f = 0.34 (1% HOAc/5% ipa/94% CH_2Cl_2).

Example 31

Boc-D-t-Bal-Phe-(Z)-cArg: As in Example 24, 0.040 g Boc-D-t-Bal-OH (0.163 mmol) and 0.077 g (0.163 mmol) Phe-(Z)cArg·HCl (Example 23) with 0.045 ml (0.326 mmol) TEA and 0.025 ml (0.163 mmol) DECP gives 0.055 g of product. R_f = 0.91 (10% MeOH/90% CH_2Cl_2).

Example 32

Boc-D-t-Bal-Phe-Arg·OAc: As in Example 6, 0.055 g (0.083 mmol) Boc-D-t-Bal-Phe-(Z)-cArg (Example 31) and 0.006 g (0.166 mmol) LAH gives 0.027 g product that is hydrogenated (0.010 g Pd/C) and lyophilized to give 0.035 g product. FAB-MS - 533 m/z (MH^+), 555 m/z $(MNa)^+$, 641 m/z $(MH + TG)^+$, 515 m/z $(MH - H_2O)^+$.

Example 33

N-Bn-N-(Z)-D-1-Adl-OH: As in Example 29, 5.20 ml (4.69 mmol) 1-adamantyl magnesium bromide, 0.495 g (2.41 mmol) cuprous bromide/dimethylsulfide complex and 0.031 g (0.963 mmol) N-Bn-N-(Z)-D-Ser-β-lactone (Example 28) in 0.514 ml methyl sulfide and 15 ml THF gives 0.323 g product. $[\alpha]_D^{25}$ = +20.3 (c = 1, chloroform). R_f = 0.25 (1% HOAC/5% ipa/94% CH_2Cl_2).

Example 34

Boc-D-1-Adl-OH: As in Example 30, 0.160 g N-Bn-N-(Z)-D-1-Adl-OH (Example 33) (0.358 mmol) and 0.032 g Pd/C gives 0.081 g product as white powder. 0.60 g of this product is protected as the Boc compound with 0.115 g di-t-butyl dicarbonate

(0.296 mmol) and 5.37 ml 1N NaOH to give 0.047 g product. $[\alpha]_D^{25}$ = +3.9 (c = 1, chloroform). R_f = 0.38 (1% HOAc/ 5% ipa/94% CH_2Cl_2).

Example 35

Boc-D-1-Adl-Phe-(Z)-cArg: As in Example 24, 0.047 g (0.145 mmol) Boc-D-1-Adl-OH (Example 34) and 0.069 g (0.145 mmol) Phe-(Z)cArg·HCl (Example 23) with 0.044 ml (0.319 mmol) TEA and 0.022 ml (0.145 mmol) DECP gives 0.067 g product. $[\alpha]_D^{25}$ = -15.8 (c = 1, chloroform). R_f = 0.64 (5% ipa/CH_2Cl_2).

Example 36

Boc-D-1-Adl-Phe-Arg-H·OAc: As in Example 6, 0.065 g Boc-D-1-Adl-Phe-(Z)-cArg (Example 35) (0.088 mmol) and 0.007 g (0.175 mmol) LAH gives 0.045 product. 0.014 g of this product is hydrogenated (0.002 g Pd/C) and lyophilized to give 0.015 g product. FAB-MS - 611 m/z (MH^+), 719 m/z $(MH + TG)^+$.

Example 37

α-N-Boc-ε-N-(Z)-D-Orn-Phe-(Z)-cArg: As in Example 24, 0.250 g (0.710 mmol) α-N-Boc-δ-N-(Z)-D-Orn-OH and 0.338 g (0.71 mmol) Phe-(Z)-cArg·HCl (Example 23) with 0.390 ml (2.80 mmol) TEA and 0.120 ml (0.800 mmol) DECP gives 0.310 g product. R_f = 0.52 (5% ipa/CH_2Cl_2).

Example 38

α-N-Adoc-ε-N-(Z)-D-Orn-Phe-(Z)-cArg: To a cold (0°C) solution of 0.300 g (0.420 mmol) of α-N-Adoc-δ-N-(Z)-D-Orn-Phe-(Z)-cArg (Example 37) in 15 ml water and 15 ml dioxane is added 0.134 g (1.60 mmol) sodium bicarbonate followed by 0.099 g (0.500 mmol) adamantyl fluoroformate in 1 ml dioxane dropwise. The cooling bath is removed, and the solution is stirred for 1 h. The dioxane is removed under vacuum, and the aqueous phase is extracted with ethyl acetate. The organic phase is washed with brine and dried with magnesium sulfate, and the solvent is removed. Chromatography on silica gel gives 0.120 g product. R_f = 0.52. (5% ipa/CH_2Cl_2).

Example 39

Adoc-D-Orn-Phe-Arg-H·OAc: As in Example 6, 0.165 g (0.190 mmol) α-N-Adoc-δ-N-(Z)-D-Orn-Phe-(Z)-cArg (Example 38) and

0.015 g (0.380 mmol) LAH gives 0.035 g product that is hydrogenated and lyophilized to give 0.030 g product.

Example 40

Adac-D-t-Bug-Phe-OBn: To a solution of 5 ml of a 15% solution of phosgene in toluene under argon is added 0.140 ml TEA (1.00 mmol). This is followed by the addition of 0.112 g (0.750 mmol) of 1-adamantanamine in 1 ml toluene dropwise via syringe. The resulting slurry is stirred for 0.3 h, and the excess phosgene is removed by purging with argon. The solid is filtered off by suction through a sintered glass funnel, and the solvent is removed. The residue is redissolved in dichloromethane and cooled to 0°C under argon. To this solution is added 0.140 ml TEA (1.00 mmol) followed by 0.100 g (0.250 mmol) D-t-Bug-Phe-OBn·HCl (see Example 16 for preparation method used to make an analogous compound) in one portion. The resulting solution is stirred for 2 h at room temperature, and the solvent is removed. Chromatography on silica gel gives 0.350 g product.

Example 41

Adac-D-t-Bug-Phe-(Z)-cArg: To a mixture of 0.082 g Adac-D-t-Bug-Phe-OH (see Example 4 for preparation method used to make an analogous compound) (0.150 mmol) and (Z)-cArg·HCl (Example 3) (0.080 g, 0.180 mmol) in 25 ml dichloromethane under argon is added 0.084 ml (0.600 mmol) TEA followed by 0.030 ml (0.200 mmol) DECP. The mixture is stirred overnight, and the solvent is removed. The residue is chromatographed on silica gel to give 0.085 g product. R_f= 0.35 (3% ipa/CH_2Cl_2).

Example 42

Adac-D-t-Bug-Phe-Arg-H: As in Example 6, 0.085 g Adac-D-t-Bug-Phe-(Z)-cArg-H (Example 41) (0.120 mmol) and 0.009 g LAH (0.240 mmol) gives 0.054 g chromatographed product that is hydrogenated and lyophilized to give 0.044 g product. FAB-MS - 596 m/z (MH^+).

Example 43

Ada-D-t-Bug-Phe-(Z)-cArg: To a solution of Boc-D-t-Bug-Phe-(Z)-cArg (see Example 5 for preparation method used to make an analogous compound) (0.060 g, 0.082 mmol) in 2 ml ethyl acetate is added 1 ml 5 M HCl in ethyl acetate, and the solution

is stirred at room temperature for 6 h. An additional 1 ml of HCl in ethyl acetate is added, and the solution is stirred overnight. The solvent is removed, and the residue is placed on the vacuum pump. The solid material is dissolved in 20 ml dichloromethane, and 0.018 g (0.091 mmol) adamantaneacetyl chloride is added. To this solution is added 0.025 ml TEA (0.180 mmol) followed by 0.014 ml DECP (0.091 mmol), and the resulting mixture is stirred overnight. Removal of the solvent and chromatography on silica gel gives 0.013 g compound. R_f= 0.52 (5% MeOH/CH_2Cl_2).

Example 44

Ada-D-t-Bug-Phe-Arg-H: As in Example 6, 0.013 g (0.018 mmol) Ada-D-t-Bug-Phe-(Z)-cArg (Example 43) and LAH (0.0015 g, 0.036 mmol) gives 0.002 g that is hydrogenated and lyophilized to afford 0.002 g product. FAB-MS - 595 m/z (MH^+), 577 m/z $(MH - H_2O)^+$.

Example 45

(4R)-3-(2-adamantyl-1-oxo)-4-(phenylmethyl)-2-oxazolidinone: To a solution of 1.00 g (6.06 mmol) of (4R)-(phenylmethyl)-2-oxazolidinone in 30 ml THF at -78°C is added 2.70 ml of butyl lithium (6.67 mmol) dropwise. After the addition is complete, the solution is stirred for 10 min, followed by the dropwise addition of 1.53 g of adamantane acetyl chloride (7.25 mmol) in 5 ml THF. After 0.25 h the cooling bath is removed, and the solution is allowed to warm to room temperature. The reaction is quenched with 2 ml water and concentrated. The residue is partitioned between ether and water, and the phases are separated. The organic phase is dried, and the solvent is removed. Chromatography with 10% ethyl acetate/petroleum ether on silica gel gives 1.62 g of the desired product as a white solid.

Example 46

(3(2R),4R)-3(2-adamantyl-2-azido-1-oxo)-4-(phenylmethyl)-2-oxazolidinone: (4R)-3-(2-adamantyl-1-oxo)-4-(phenylmethyl)-2-oxazolidinone (Example 45) (0.389 g, 1.10 mmol) is dissolved in 15 ml THF and cooled to -78°C. This solution is then added via cannula to a 0.3 M THF solution of 1.27 mmol of KHMDS that is also maintained at -78°C. After stirring for 0.5 h at this

temperature, a 78°C solution of trisyl azide (see Harmon, R.E., G. Wellman & S.K. Gupta, "The Reaction of Arylsulfonyl Azides with N-Methylindole", Journal of Organic Chemistry, Vol. 38, No. 1 (1973), pp. 11-16) (0.420 g, 1.37 mmol) in 5 ml THF is added via cannula. After 2 min, 5 equivalents of acetic acid is added, and the solution is allowed to warm to room temperature overnight. The solution is diluted with dichloromethane and washed with brine and saturated sodium bicarbonate. After drying (magnesium sulfate) and filtration, the solvent is removed. The residue is chromatographed on silica gel to afford 0.340 g (0.860 mmol) of the desired product as a colorless oil.

Example 47

(2R)-2-azido-adamantaneacetic acid: (3(2R),4R)-3(2-adamantyl-2-azido-1-oxo)-4-(phenylmethyl)-2-oxazolidinone (Example 46) (0.270 g, 0.690 mmol) is dissolved in 10 ml THF and cooled to 0°C. To this solution is added a mixture of 3.63 ml of 0.2 M lithium hydroxide, 0.35 ml of 30% hydrogen peroxide and 4 ml of water dropwise. After 1 h at 0°C, 10 equivalents of sodium bisulfite in 2 ml water is added, and the solution is stirred for 15 min. The THF is removed, and the remaining aqueous solution is extracted 3 times with ethyl acetate; the organic phase is dried with magnesium sulfate. After filtration, the solvent is removed, and the residue is chromatographed on silica gel to afford 0.135 g of the desired product as a white solid.

Example 48

(R)-Adoc-Adg-OH: (2R)-2-azido-adamantaneacetic acid (Example 47) (0.070 g, 0.340 mmol) is dissolved in 2:1 acetic acid/water (2 ml), and 0.150 g Pd/C is added. The slurry is hydrogenated on the Paar® apparatus at 50 psi for 16 h. The slurry is filtered through Celite®, and the solvent is removed. The residue is dissolved in 0.34 ml 1N NaOH and 2 ml water, and 2 ml dioxane is added. To this solution is added 0.027 g sodium bicarbonate, followed by addition of adamantyl fluoroformate (0.101 g, 0.510 mmol) in 1 ml dioxane. After 0.5 h, the cooling bath is removed, and the solution is warmed to room temperature. The mixture is diluted with ethyl acetate, and the phases

separated. The aqueous phase is acidified to pH=2 and extracted with ethyl acetate. Removal of solvent gives 0.040 g product.

Example 49

Adoc-D-Adg-Phe-(Z)-cArg: As in Example 24, 0.040 g (0.100 mmol) of (R)-Adoc-Adg-OH (Example 48) and 0.049 g (0.100 mmol) of Phe-(Z)-cArg·HCl (Example 23) with 0.056 ml (0.400 mmol) TEA and 0.018 ml (0.120 mmol) DECP gives 0.031 g of product after chromatography. R_f= 0.65 (5% ipa/CH_2Cl_2).

Example 50

Adoc-D-Adg-Phe-Arg-H: As in Example 6, 0.030 g Adoc-D-Adg-Phe-(Z)-cArg (Example 49) (0.037 mmol) and LAH (0.003 g, 0.075 mmol) gives 0.010 g chromatographed product that is hydrogenated with 0.002 g Pd/C and lyophilized to afford 0.009 g product. FAB-MS 675 m/z (MH^+).

Example 51

Adoc-D-t-Bug-Phe-(Z)-Lys-OMe: To a solution of 0.432 g Adoc-D-t-Bug-Phe-OH (see Example 17 for preparation method used to make an analogous compound) (0.946 mmol) and 0.298 g N-ε-(Z)-Lys-OMe (0.901 mmol) in 25 ml dichloromethane is added 0.276 ml TEA (1.98 mmol) followed by 0.144 ml DECP (0.946 mmol); the solution is stirred overnight. The solvent is removed, and the residue is chromatographed on silica gel to give 0.495 g product. R_f = 0.49 (4% MeOH/CH_2Cl_2).

Example 52

Adoc-D-t-Bug-Phe-(Z)-Lysinol: Adoc-D-t-Bug-Phe-(Z)-Lys-OMe (Example 51) (0.070 g, 0.096 mmol) is dissolved in 1 ml of ethanol and cooled in an ice/methanol bath. To this solution is added solid anhydrous calcium chloride (0.021 g, 0.191 mmol) and 1 ml THF. Sodium borohydride (0.144 g, 0.382 mmol) is added in one portion and the resulting slurry is allowed to warm to room temperature over a period of 3 h. The reaction is quenched with 1 ml of 1 M citric acid. The solvent is removed, and the residue is partitioned between ethyl acetate and water. The organic phase is washed with brine and dried, and the solvent is removed. Chromatography on silica gel affords 0.042 g of the desired compound. $[\alpha]_D^{25}$ = +14.8 (c=1, EtOH).

Example 53

Adoc-D-t-Bug-Phe-(Z)-Lys-H: To a solution of 0.026 ml oxalyl chloride (0.275 mmol) in 5 ml dichloromethane at -78°C is added 0.042 ml DMSO (0.570 mmol) in 0.500 ml dichloromethane dropwise. After 5 min, 0.160 g Adoc-D-t-Bug-Phe-(Z)-Lys-OMe (Example 51) (0.227 mmol) in 1 ml dichloromethane is added. The solution is stirred at -78°C for 0.3 h and 0.158 ml TEA is added. The solution is then warmed to 0°C and quenched with 2 ml water. The mixture is washed with 1N HCl, saturated sodium bicarbonate and brine. The organic phase is dried and filtered, and the solvent is removed. Chromatography on silica gel of the residue gives 0.096 g of the desired product. $[\alpha]_D^{25}$ = -45.3 (c=1,$CHCl_3$).

Example 54

Adoc-D-t-Bug-Phe-Lys-H·TFA: To a solution of 0.009g Adoc-D-t-Bug-Phe-(Z)-Lys-H (Example 53) (0.013 mmol) and 0.006 g of 9.7% HCl in dioxane in 0.900 ml DMF is added 0.005 g Pd black. This slurry is hydrogenated at atmospheric pressure for 0.3 h. The slurry is diluted with 1 ml dioxane and filtered through Celite®. The filter cake is washed with methanol, and the solvent is removed. The residue is chromatographed on reverse phase (water/acetonitrole/0.1% TFA) to give 0.008 g of the desired product. FAB-MS - 569 m/z (MH^+), 677 m/z $(MH + TG)^+$.

Example 55

eBroc-D-t-Bug-Phe-(Z)-cArg: To a solution of 0.080 g of (-)endoborneol (0.520 mmol) and 0.041 ml pyridine (0.520 mmol) in 2 ml toluene is added 7 ml of a 12% solution of phosgene in toluene dropwise. After 1 h, argon is bubbled through the solution to purge any remaining phosgene. After 10 min the slurry is filtered, and the solvent is removed. The residue is dissolved in dichloromethane, and 0.127 g of D-t-Bug-Phe-(Z)-cArg·HCl (see Example 20 for preparation method used to make an analogous compound) (0.240 mmol) is added followed by 0.100 ml (0.720 mmol) of TEA. The solution is stirred overnight, and the solvent is removed. Chromatography on silica gel gives 0.100 g of the desired product. R_f (5% ipa/CH_2Cl_2) = 0.75.

Example 56

eBroc-D-t-Bug-Phe-Arg-H: As in Example 6, 0.090 g (0.120 mmol) of eBroc-D-t-Bug-Phe-(Z)-cArg (Example 55) and 0.009 g LAH (0.240 mmol) gives 0.065 g product after chromatography. This compound is hydrogenated on the Paar® apparatus for 3 h (AcOH/MeOH - 1/20) to give 0.045 g after lyophilization. FAB-MS - 599 m/z (MH^+), 707 m/z $(MH + TG)^+$.

Example 57

Tr-p-Nph-OMe: 26.0 g (99.8 mmol) of p-Nph-OMe·HCl is suspended in 500 ml dichloromethane, and 27.8 ml TEA (199.6 mmol) is added dropwise. After the addition is complete, 26.4 g (94.8 mmol) of trityl chloride in 250 ml dichloromethane is added. The yellow solution is stirred for 2 h, washed with brine, dried with magnesium sulfate and filtered, the solvent is removed to give 44.0 g of product that is of suitable purity to be used in the reaction of Example 58.

Example 58

Tr-p-nitrophenylalaninol: To a solution of 45.3 g (97.2 mmol) of Tr-p-Nph-OMe (Example 57) in 500 ml THF at 0°C under argon is added 200 ml (300 mmol) DIBAL-H at a moderate rate. After 1 h, the solution is transferred to a 2 l Erlenmeyer flask and cooled to 0°C. To this solution is added 500 ml saturated potassium sodium tartrate solution cautiously. The mixture is extracted with 3 X 1 l ethyl acetate, and the organic phases are dried. Removal of solvent gives 40 g that is chromatographed on silica gel to give 26.9 g of product.

Example 59

Tr-p-nitrophenylalaninal: To a solution of 0.581 ml oxalyl chloride (6.67 mmol) in 50 ml dichloromethane under argon at -78°C is added 0.946 ml DMSO (13.3 mmol) in 2 ml dichloromethane dropwise. After the addition is complete, 2.25 g (5.13 mmol) of Tr-p-nitrophenylalaninol (Example 58) in 7 ml dichloromethane is added. The solution is stirred for 0.5 h and is then poured into a mixture of ether and water. The phases are separated, and the organic phase is washed with water, dried with magnesium sulfate and filtered; the solvent is removed. The residue is chromatogr-

aphed on silica gel to afford 2.00 g product. R_f= 0.55 (2% $CH_3C(O)CH_3/CH_2Cl_2$), $[\alpha]_D^{25}$ = +67.8 (c=1,CH_2Cl_2).

Example 60

4-p-Nitrophenyl-1,1,1-trifluoro-3-tritylamino-2-butanol: To a solution of 2.23 g Tr-p-nitrophenylalaninol (Example 59) (5.11 mmol) in 100 ml THF under argon is added 0.951 g CF_3TMS (Scheme D) (6.13 mmol) followed by the addition of 0.163 g tetrabutylammonium fluoride trihydrate (0.510 mmol); the solution is stirred for 2 h. The solvent is removed, and the residue is chromatographed on silica gel to afford 1.73 g of product. R_f = 0.50 (2% $CH_3C(O)CH_3/CH_2Cl_2$).

Example 61

3-Amino-4-p-aminophenyl-1,1,1-trifluoro-2-butanol: To a solution of 4-p-Nitrophenyl-1,1,1-trifluoro-3-tritylamino-2-butanol (Example 60) (1.00 g, 1.98 mmol) in 50 ml ethanol is added 2.23 g (9.88 mmol) tin chloride dihydrate. The mixture is refluxed for 1 h and poured into ice water (100 ml). The pH is adjusted to 8.4 with sodium bicarbonate, and the mixture is extracted with ethyl acetate (2 X 250 ml). The organic phase is dried and filtered, and the solvent is removed. Chromatography on silica gel gives 0.800 g product. R_f= 0.18, 0.30 (1% NH_4OH/10% MeOH/89% CH_2Cl_2).

Example 62

4-p-Aminophenyl-3-t-butyloxycarbonylamino-1,1,1-trifluoro-2-butanol: To a solution of 3-amino-4-p-aminophenyl-1,1,1-trifluoro-2-butanol (Example 61) (5.26 g, 22.5 mmol) in 200 ml THF is added 5.31 g di-t-butyl dicarbonate (23.6 mmol), and the solution is stirred overnight. The solvent is removed, and the residue is chromatographed on silica gel to give 5.70 g product. R_f = 0.48, 0.68 (1% NH_4OH/1% ipa/98% CH_2Cl_2).

Example 63

3-t-Butyloxycarbonylamino-4-di-CBz-p-guanidinophenyl-1,1,1-trifluoro-2-butanol: To a solution of 0.020 g (0.060 mmol) of 4-p-aminophenyl-3-t-butyloxycarbonylamino-1,1,1-trifluoro-2-butanol (Example 62) in 5 ml THF under argon is added 0.027 mg (0.072 mmol) of N,N',-di-Cbz-S-methylisothiourea (Example 74) followed by addition of 5.7 mg mercuric acetate. The mixture is

stirred overnight, and an additional 5.7 mg of mercuric acetate is added the following morning. After 1 h, the THF is evaporated and the residue is redissolved in ethyl acetate. The organic phase is washed with saturated sodium bicarbonate, 0.1N HCl and brine. The solution is dried and filtered, and the solvent is removed. Chromatography on silica gel gives 0.035 g product. R_f= 0.28 (3% ipa/CH_2Cl_2).

Example 64

3-Amino-4-di-Cbz-p-guanidinophenyl-1,1,1-trifluoro-2-butanol hydrochloride salt: A solution of 2.8 g (4.34 mmol) of 3-t-butyloxycarbonylamino-4-di-Cbz-p-guanidinophenyl-1,1,1-trifluoro-2-butanol (Example 63) in 40 ml dioxane and 15 ml 4M HCl in dioxane is stirred for 2 h at room temperature. The dioxane is removed in vacuo, and the residue is triturated with ether. The slurry is filtered under argon and is found to weigh 2.4 g.

Example 65

3-(Adamantyloxy-D-t-butylglycylphenylalanyl)amino-4-di-Cbz-p-guanidinophenyl-1,1,1-trifluoro-2-butanol: To a solution of 0.750 g 3-amino-4-di-Cbz-p-guanidinophenyl-1,1,1-trifluoro-2-butanol (Example 64) (1.29 mmol) and 0.647 g Adoc-D-t-Bug-Phe-OH (see Example 17 for preparation method used to make an analogous compound) (1.42 mmol) in 10 ml DMF is added 0.500 ml TEA (3.58 mmol) followed by 0.232 ml DECP (1.42 mmol); the mixture is stirred overnight. The solvent is removed and the residue is chromatographed (40% ethyl acetate/petroleum ether) on silica gel to afford 0.890 g product. R_f = 0.70 (5% ipa/CH_2Cl_2).

Example 66

3-(Adamantyloxy-D-t-butylglycylphenylalanyl)amino-4-di-Cbz-p-guanidinophenyl-1,1,1-trifluoro-2-butanone: To a solution of 0.452 g Dess-Martin periodinane (1.07 mmol) in 10 ml dichloromethane is added 0.352 g 3-(adamantyloxy-D-t-butylglycylphenylalanyl)amino-4-di-Cbz-p-guanidinophenyl-1,1,1-trifluoro-2-butanol (Example 65) (0.356 mmol) in 10 ml dichloromethane dropwise. The red-brown solution is stirred for 2 h and is diluted with 30 ml ether. The mixture is poured into 50 ml saturated sodium bicarbonate containing 1.85 g sodium thiosulfate. This solution is stirred for 10 min, and 30 ml ether is added. The phases are

separated, and the organic phase is dried with magnesium sulfate. The solvent is removed, and the residue is chromatographed on silica gel to give 0.120 g product. R_f= 0.52 (75% EtOAc/pentane), $[\alpha]_D^{25}$ = -32.3 (c=1,CH_2Cl_2).

Example 67

3-(Adamantyloxy-D-t-butylglycylphenylalanyl)amino-4-p-guanidinophenyl-1,1,1-trifluoro-2-butanone: To a solution of 0.120 g 3-(adamantyloxy-D-t-butylglycylphenylalanyl)amino-4-di-Cbz-p-guanidinophenyl-1,1,1-trifluoro-2-butanone (Example 66) (0.122 mmol) in 20 ml methanol and 0.5 ml acetic acid is added 0.025 g Pd/C, and the slurry is hydrogenated at 45 psi for 1.5 h. the slurry is filtered through Celite®, and the solvent is removed. Reverse phase HPLC (acetonitrile/water/TFA) gives 0.017 g of one diastereomer and 0.028 g of the other. FAB-MS - 732 m/z ($MH^+ + H_2O$), 746 m/z ($MH^+ + CH_3OH$).

Example 68

4-t-Butyloxycarbonylamino-1-nitrobutane: To a solution of 4.00 g 4-t-butyloxyamino-1-aminobutane (see Stahl, G.L., R. Walter & C.W. Smith, "General Procedure for the Synthesis of Mono-N-acylated 1,6-Diaminohexanes", Journal of Organic Chemistry, Vol. 43, No. 11 (1978), pp. 2285-2286) (21.3 mmol) in 50 ml dichloroethane is added 14.7 g (85.0 mmol) MCPBA in 1 g portions. The temperature is raised to reflux (83°C) and held there for 3 h. An additional 7.80 g (45.0 mmol) of MCPBA is added and the reflux is continued for another hour. The solution is cooled and washed with 3 X 30 ml 1N NaOH. The organic layer is dried (magnesium sulfate), and the solvent is removed. Chromatography on silica gel gives 1.90 g product.

Example 69

6-Boc-amino-3-nitro-1,1,1-trifluoro-2-hexanol: To a solution of 0.500 g (2.29 mmol) 4-t-Boc-amino-1-nitrobutane (Example 68) and 0.239 g (2.08 mmol) of trifluoroacetaldehyde hydrate in 10 ml THF is added 0.013 g crushed potassium carbonate. The slurry is heated to 50°C and stirred at this temperature overnight. The slurry is dissolved in ether and washed with brine. The organic phase is dried, and the solvent is

removed. Chromatography of the residue gives 0.380 g of product as a mixture of diastereomers. R_f = 0.34 (5% ipa/CH_2Cl_2).

Example 70

3-Amino-6-Boc-amino-1,1,1-trifluoro-2-hexanol: To a solution of 0.280 g (0.885 mmol) of 6-Boc-amino-3-nitro-1,1,1-trifluoro-2-hexanol (Example 69) in 5 ml methanol is added 5 drops of acetic acid and 0.056 g 10% Pd/C. The slurry is hydrogenated at 45 psi for 20 h and filtered through Celite®. The solvent is removed, and the residue is chromatographed on silica gel to give two diastereomers (0.072 g and 0.108 g) that are easily separated. R_f= 0.27, 0.45 (1% NH_4OH/2.5% MeOH/96.5% CH_2Cl_2).

Example 71

3-(eBroc-D-t-Bug-Phe-amino-6-t-butyloxycarbonylamino-1,1,1-trifluoro-2-hexanol: To a solution of 3-amino-6-t-Boc-amino-1,1,1-trifluoro-2-hexanol (Example 70) (0.600 g, 1.31 mmol) and eBroc-D-t-Bug-Phe-OH (see Example 17 for preparation method used to make an analogous compound) (0.370 g, 1.31 mmol) in 20 ml dichloromethane under argon is added 0.360 ml TEA (2.62 mmol) followed by the addition of 0.200 ml DECP (1.31 mmol). The mixture is stirred overnight, and the solvent is removed. The residue is chromatographed on silica gel to afford 0.800 g product. R_f= 0.34 (5% ipa/CH_2Cl_2).

Example 72

3-(eBroc-D-t-Bug-Phe)-amino-6-guanidino-1,1,1-trifluoro-2-hexanol TFA salt: To a solution of 0.800 g 3-(eBroc-D-t-Bug-Phe)-amino-6-Boc-amino-1,1,1-trifluoro-2-hexanol (Example 71) (1.10 mmol) in 20 ml dioxane is added 3 ml 3.2M HCl in dioxane; the mixture is stirred for 2 h. At this time an additional 1 ml HCl is added, and the mixture is stirred for an additional 2.5 h. The solvent is removed, and the residue is placed on the vacuum pump overnight. The solid material is dissolved in 50 ml dry DMF, and the pH is adjusted to 8.5 with TEA. In a separate flask 7.6 g (37.8 mmol) of 3,5-dimethylpyrazole-1-carboxamidine nitrate (DMPCN) is dissolved in 5 ml DMF, and the pH adjusted to 8.5. The two solutions are combined and stirred for 5 days. The pH is adjusted to 5.8 with acetic acid, and the solvent is removed.

Reverse phase HPLC purification of the residue gives 0.190 g of the desired product.

Example 73

3-(eBroc-D-t-Bug-Phe)-amino-6-guanidino-1,1,1-trifluoro-2-hexanone TFA salt: To a solution of Dess-Martin periodinane (0.400 g, 0.850 mmol) in 10 ml CH_2Cl_2 is added a solution of 3-(eBroc-D-t-Bug-Phe)-amino-6-guanidino-1,1,1-trifluoro-2-hexanol TFA salt (Example 72) (0.190 g, 0.240 mmol) in 10 ml CH_2Cl_2. The mixture is stirred overnight, and the solvent is removed. The residue is redissolved in CH_2Cl_2 and washed with saturated $NaHCO_3$ solution. The solvent is removed from the organic phase, and the residue is dissolved in 2 ml MeOH and passed through a column of Biorad-AGB1X8/200-400 mesh - acetate form. After removing the solvent the residue is chromatographed (reverse phase HPLC) to afford 0.080 g product.

Example 74

N,N'-bis-carbobenzyloxy-S-methyl-isothiourea: To a solution of S-methyl-isothiourea sulfate dimer (5.00 g, 18.0 mmol) of cold (0°C) 1N NaOH (36.0 ml, 36.0 mmol) is added 11.3 ml (79.0 mmol) benzyl chloroformate and 79.0 ml (79.0 mmol) 1N NaOH dropwise from separate addition funnels. The slurry is stirred for 0.5 h at 0°C and 1.5 h at room temperature. The solution is extracted with ethyl acetate, dried with magnesium sulfate and the filtrate is evaporated. Chromatography on silica gel gives 0.262 g product. R_f = 0.40 (15% EtOAc/pet ether).

Example 75

Boc-Cha-OBn: To a slurry of Boc-Cha-OH (1.56 g, 5.75 mmol) and $NaHCO_3$ (1.54 g, 18.33 mmol) in 50 ml DMF is added 0.700 ml (6.72 mmol) benzyl bromide. The mixture is stirred for 2 days. The DMF is evaporated, and the solid is dissolved in EtOAc. The slurry is washed with water; the organic phase is dried, and the filtrate is evaporated. 1.91 g product that is homogeneous by TLC is recovered. R_f = 0.70 (25% EtOAc/pet ether).

Example 76

Cha-OBn·HCl: To a solution of Boc-Cha-OBn (1.81 g, 5.01 mmol) (Example 75) in dioxane is added 18 ml of 3.34M HCl in dioxane. After 1 h, 10 ml additional 3.34M HCl in dioxane is

added. The solvent is removed, and the residue is triturated with EtOAc, filtered and placed on a vacuum pump. 1.22 g of a white solid is recovered.

Methods for Testing Activity of the Compounds

The following non-limiting procedures are methods for testing the anti-inflammatory and/or analgesic activity of the tripeptide derivatives of the subject invention.

Several enzyme inhibition assays are known to be predictive of anti-inflammatory activity for compounds. Such enzyme assays are useful for measuring the activity of compounds of the subject invention. Such enzyme assays include the following: porcine pancreatic kallikrein (PPK) - see references A, E and F; human urinary kallikrein (HUK) - see references E and F; human plasma kallikrein - see references B and E; human plasmin (HP) - see references B and C; and urokinase (UK) - see reference D. The indicated references, which are hereby incorporated herein by reference, are the following:

(A) Lottenberg, R., U. Christensen, C.M. Jackson & P.L. Coleman, "Assay of Coagulation Proteases Using Peptide Chromogenic and Fluorogenic Substrates", Methods in Enzymology, Vol. 80, Academic Press, New York, NY (1981), pp. 341-361; (B) Geiger, R. "Kallikrein", Methods of Enzymatic Analysis, Vol. V, 3rd Edition, Bergmeyer, ed., Academic Press, New Nork, NY (1984), pp. 129-143; (C) Morris, J.P., S. Blatt, J.R. Powell, D.K. Strickland & F.J. Castellino, "Role of Lysine Binding Regions in the Kinetic Properties of Human Plasmin", Biochemistry, Vol. 20, No. 17 (August 18, 1981) p. 4811; (D) Wohl, R.C., L. Summaria & K.C. Robbins, "Kinetics of Activation of Human Plasminogen by Different Activator Species at pH 7.4 and 37°C" The Journal Biological Chemistry, Vol. 255, No. 5 (March 10, 1980), pp. 2005-2013; (E) Okunishi, H., J. Burton & J. Spragg, "Specificity of Substrate Analogue Inhibitors of Human Urinary Kallikrein", Hypertension, Vol. 7, No. 3, Suppl. I (May-June, 1985), pp. I-72-I75; (F) Amundsen, E., J. Putter, P. Friberger, M. Knos, M. Larsbraten & G. Claeson, "Methods for the Determination of Glandular Kallikrein by Means of a Chromogenic Tripeptide

Substrate", In Kinins-II part A, Fuji, S., et al., eds., Plenum Press, New York, NY (1979) pp. 83-95.

Another useful assay of activity is based on a method for determination of slow-binding enzyme inhibition disclosed in Imperiali, B. & R.H. Abeles, "Inhibition of Serine Proteases by Peptidyl Fluoromethyl Ketones", Biochemistry, Vol. 25 (1986) pp. 3760-3767. The method is modified as described below in order to study the slow binding inhibition of human plasmin (A*) and kallikrein (pig pancreatic) (B*).

Reactions: (A*) The reaction mixtures contain 78 mM tris-HCl buffer, pH 7.4, 78 mM NaCl, 0.2 mg/ml bovine serum albumin, 0.2 mM S-2251 (D-Val-Leu-Arg-p-nitroanilide), 0.5 U/ml plasmin (1 μM), and variable concentrations of the test compound to be studied, in a total volume of 1 ml. The stock solution of plasmin is 1 U/ml in 50% glycerol. The absorbance change due to release of p-nitroaniline on enzymatic cleavage of S-2251 is monitored using an HP-8450 spectrophotometer system, set to measure $A^{400-410} - A^{470-490}$. The temperature is 30°C.

Calculations: K_{obsd} and v_s (steady state inhibited rate) are determined by fitting the progressive curve (first 20 min) to the integrated rate equation (i) using Labtech Notebook® software. Estimated k_i is calculated for each run from v_s and the uninhibited rate v (equation ii), with (S) = 0.2 mM and K_m = 0.77 mM.

(i) $A = v_s t - ((v_s - v_o)/k_{obsd})(1 - \exp(-k_{obsd}t)) + A_o$

(ii) $k_i = (I)/((v/v_s-1)(1 + (s)/k_m))$

A plot of k_{obsd} vs test compound concentration for the different runs is then fit to a line, $y = mx + b$ (see equation iii); $k_{on} = m(1 + (S)/k_m)$ and $k_{off} = b$ are then calculated. Finally k_i is calculated from equation iv.

(iii) $k_{obsd}=(I)/((v/v_s-1)(1 + (S)/k_m))$

(iv) $k_i = k_{off}/k_{on}$

Several in vivo assays are known to be predictive of the anti-inflammatory activity of compounds. Such in vivo assays are useful for measuring the activity of compounds of the subject invention. Such in vivo assays are disclosed in the following references which are hereby incorporated herein by reference:

Winter, C.A., E.A. Risley, G.V. Nuss, "Carrageenin-Induced Edema in Hind Paw of the Rat as an Assay for Antiinflammatory Drugs", Proc. Soc. Exp. Biol., N.Y., Vol. 111 (1962), pp. 544-547; Vander Wende, C. & S. Margolin, "Analgesic Tests Based Upon Experimentally Induced Acute Abdominal Pain in Rats", Federal Proceedings., Vol. 15 (1956), p. 494; Blackham, A., J.W. Burns, J.B. Farmer, H. Radziwonik, J. Westwick, "An X-Ray Analysis of Adjuvant Arthritis in the Rat. The Effect of Prednisolone and Indomethacin", Agents and Actions, Vol. 7/1 (1977), p. 145-151; Winter, C.A. & G.W. Nuss, "Treatment of Adjuvant Arthritis in Rats with Anti-Inflammatory Drugs" Arthritis and Rheumatism, Vol. 9, No. 3 (June, 1966), pp. 394-404; and Francis, M.D., K. Hovancik & R.W. Boyce, "NE-58095: A Diphosphonate Which Prevents Bone Erosion and Preserves Joint Architecture in Experimental Arthritis", Int. J. Tiss. Reac., Vol. XI, No. 5 (1989), pp. 239-252.

Compositions and Methods of Using the Compounds

The following non-limiting examples exemplify contemplated compositions and uses for the subject invention.

Example 77

Tablets are made by conventional procedures, each having the following composition:

Component	Quantity (mg)
Ipoc-D-t-Bug-Phe-Arg-CF_3	400
Microcrystalline cellulose	200
Pregelatinized starch	200
Povidone K-30	40
Magnesium stearate	20

One tablet is administered orally four times daily to a patient to aleviate inflammation in joints due to arthritis.

Example 78

A lotion is made by conventional procedures, the lotion having the following composition:

Component	Quantity (%)
Adoc-D-Val-Phe-Arg-H	2.5
Glycerin	4.0
Methyl paraben	0.2

Propyl paraben	0.1
Carbopol 934	0.15
NaOH	0.46
Cetyl stearyl palmitate	1.0
Stearic acid	0.5
Lanolin fatty acids	0.5
Cetyl alcohol	3.0
Zantham gum	0.3
Sodium stearoyl-2-lactolate	0.75
Isopropyl myristate	2.0
Water	q.s.

One gram of the lotion is administered topically to the skin in the area of a burn twice daily to reduce inflammation and pain.

Example 79

A solution is made by conventional means, each 2 ml of solution having the following composition:

Component	Quantity (mg)
eBroc-D-t-Bug-Phe-Arg-H	80
Benzalkonium chloride	40
Sterile aqueous saline solution	q.s.

A 2 ml dose of the solution is injected intramuscularly to a patient with arthritis to reduce inflammation and pain.

Example 80

A solution is made by conventional means, the solution having the following composition:

Component	Quantity (%)
Adoc-D-t-Bug-Phe-Arg-H	5.0
Benzalkonium chloride	0.02
Sodium carboxymethyl cellulose	0.01
Aqueous saline solution	q.s.

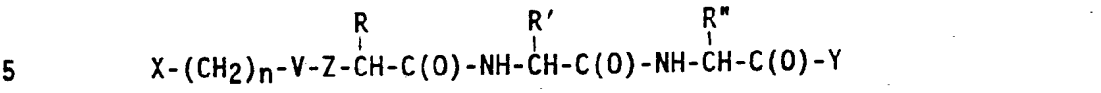
A 0.2 ml dose of the solution is administered by inhalation to a patient as needed to alleviate upper respiratory distress due to asthma.

While particular embodiments of the subject invention have been described, it will be obvious to those skilled in the art that various changes and modifications of the subject invention

can be made without departing from the spirit and the scope of the invention. It is intended to cover, in the appended claims, all such modifications that are within the scope of this invention.

Claims:

1. A compound, and the pharmaceutically-acceptable salts and hydrates thereof, having the structure:



wherein

(a) n is an integer of from 0 to 2;

(b) -R is selected from straight or branched alkyl, saturated or unsaturated with 1 or 2 double bonds, having from 1 to 6 carbon atoms; and cyclic alkyl, saturated or unsaturated with 1 or 2 double bonds, having from 3 to 13 carbon atoms; and the carbon atom to which -R is bonded is in either D or L configuration;

(c) -R′ is selected from branched alkyl, saturated or unsaturated with 1 or 2 double bonds, having from 3 to 6 carbon atoms; cyclic alkyl, saturated or unsaturated with 1 or 2 double bonds, having from 3 to 13 carbon atoms; and arylalkyl, the alkyl portion being saturated and having from 1 to 3 carbon atoms; and the carbon atom to which -R′ is bonded is in L configuration;

(d) -R" is $-(CH_2)_m$-A-NH_2 or $-(CH_2)_m$-A-B-$C(NH_2)$=NH, wherein m is an integer of from 1 to 5, -A- is a covalent bond, or p-phenyl or p-cyclohexyl, and -B- is a covalent bond or -NH-; and the carbon atom to which -R" is bonded is in L configuration;

(e) -Y is hydrogen or trifluoromethyl;

(f) -Z- is -O- or -NH-;

(g) -V- is selected from -OC(O)-, -N(Q)C(O)-, -N(Q)C(S)-, -C(O)-, $-SO_2$- and -P(O)(OH)-; when -V- is -OC(O)-, -Z- is -NH-;

(h) -X is selected from cyclic alkyl, branched alkyl having at least two branches, and aryl, each having from 5 to 20 carbon atoms; except that when -V- is -OC(O)-, n is 0, and -Y is -H, -X is other than t-butyl; and

(i) -Q is selected from hydrogen; and straight or branched chain alkyl, saturated or unsaturated with 1 or 2

double bonds, having from 1 to 6 carbon atoms; or -Q and -X are covalently linked forming a cyclic moiety which includes the nitrogen to which -Q is bonded and from 5 to 20 carbon atoms.

2. The compound of Claim 1 wherein all alkyl portions of -R, -R′ and -X are saturated, the alkyl portion of all aryl alkyl are saturated and have from 1 to 3 carbon atoms, and all alkyl portions of -R′ are unsubstituted.

3. The compound of Claim 1 or 2 wherein -V- is selected from -OC(O)-, -N(Q)C(O)-, -N(Q)C(S)-, and -C(O)-, preferably from -OC(O)- and -NHC(O)-; all aryl portions of -R and -R′ are unsubstituted; n is 0 or 1; and all alkyl and aryl portions of -X are unsubstituted.

4. The compound of any of Claims 1-3 wherein -Q is hydrogen; the carbon to which -R is bonded is in D configuration; -Z- is -NH-; and -A- is p-phenyl or p-cyclohexyl and m is 1, or -A- is a covalent bond, -B- is -NH-, and m is 2-4.

5. The compound of any of Claims 1-4 wherein -X is cyclic alkyl or aryl, preferably cyclic alkyl having from 8 to 15 carbon atoms, and -Y is preferably $-CF_3$.

6. A compound, and the pharmaceutically-acceptable salts and hydrates thereof, having the structure:

$$X'\text{-}AA^1\text{-}AA^2\text{-}AA^3\text{-}Y$$

wherein

(a) $-AA^1-$ is selected from t-butylglycyl, valyl, isoleucyl, leucyl, cyclohexylglycyl, cyclohexylalanyl, phenylalanyl, naphthylalanyl, tryptophyl and adamantylglycyl;

(b) $-AA^2-$ is selected from phenylalanyl, naphthylalanyl, cyclohexylalanyl, leucyl and isoleucyl;

(c) $-AA^3-$ is selected from arginyl, lysyl and p-guanidinophenylalanyl, preferably arginyl;

(d) -X′ is selected from t-butyloxycarbonyl, adamantyloxycarbonyl, adamantoyl, fluorenylmethoxycarbonyl, adamantaneacetyl, adamantylaminocarbonyl, morphilinoyl, noradamantyl, homoadamantyloxycarbonyl, isopinocamphanyloxycarbonyl, 3,5-dimethyladamantyloxycarbonyl, endo-bornyloxycarbonyl, naphthyloxycarbonyl and menthyloxycarbonyl; and

(e) -Y is hydrogen or trifluoromethyl; except that when -Y is hydrogen, -X′ is other than t-butyloxycarbonyl.

7. The compound of Claim 6 wherein

(a) -AA^1- is selected from t-butylglycyl, valyl and isoleucyl;

(b) -AA^2- is phenylalanyl;

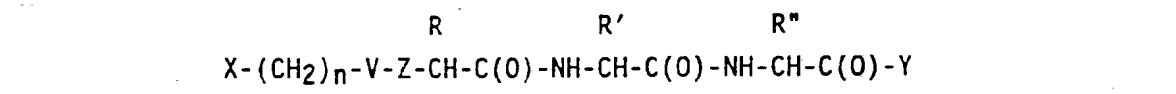
(c) -AA^3- is arginyl; and

(d) -X′ is selected from adamantyloxycarbonyl, isopinocamphanyloxycarbonyl and endo-bornyloxycarbonyl, preferably adamantyloxycarbonyl;

preferably the compound having the structure Adoc-D-t-Bug-Phe-Arg-H.

8. A pharmaceutical composition comprising:

(1) a compound, and the pharmaceutically-acceptable salts and hydrates thereof, having the structure:



wherein

(a) n is an integer of from 0 to 2;

(b) -R is selected from straight or branched alkyl, saturated or unsaturated with 1 or 2 double bonds, having from 1 to 6 carbon atoms; and cyclic alkyl, saturated or unsaturated with 1 or 2 double bonds, having from 3 to 13 carbon atoms; and the carbon atom to which -R is bonded is in either D or L configuration;

(c) -R′ is selected from branched alkyl, saturated or unsaturated with 1 or 2 double bonds, having from

3 to 6 carbon atoms; cyclic alkyl, saturated or unsaturated with 1 or 2 double bonds, having from 3 to 13 carbon atoms; and arylalkyl, the alkyl portion being saturated and having from 1 to 3 carbon atoms; and the carbon atom to which -R' is bonded is in L configuration;

(d) -R" is $-(CH_2)_m-A-NH_2$ or $-(CH_2)_m-A-B-C(NH_2)=NH$, wherein m is an integer of from 1 to 5, -A- is a covalent bond, or p-phenyl or p-cyclohexyl, and -B- is a covalent bond or -NH-; and the carbon atom to which -R" is bonded is in L configuration;

(e) -Y is hydrogen or trifluoromethyl;

(f) -Z- is -O- or -NH-;

(g) -V- is selected from -OC(O)-, -N(Q)C(O)-, -N(Q)C(S)-, -C(O)-, $-SO_2-$ and -P(O)(OH)-; when -V- is -OC(O)-, -Z- is -NH-;

(h) -X is selected from cyclic alkyl, branched alkyl having at least two branches, and aryl, each having from 5 to 20 carbon atoms; and

(i) -Q is selected from hydrogen; and straight or branched chain alkyl, saturated or unsaturated with 1 or 2 double bonds, having from 1 to 6 carbon atoms; or -Q and -X are covalently linked forming a cyclic moiety which includes the nitrogen to which -Q is bonded and from 5 to 20 carbon atoms; and

(2) a pharmaceutically-acceptable carrier.

9. A pharmaceutical composition comprising a compound of any of Claims 1-7 and a pharmaceutically-acceptable carrier.

10. Use of a compound of any of Claims 1-7 for manufacture of a medicament for treating inflammation or pain.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 92/08901

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07K5/06; A61K37/64

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07K ; A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	FR,A,2 490 632 (NIPPON KAYAKU KABUKISHI KAISHA) 26 March 1982 see the whole document ---	1-10
X	EP,A,0 195 212 (MERRELL DOW PHARMACEUTICALS INC.) 24 September 1986 see the whole document, in particular the first compound mentioned on page 21 and the second compound mentioned on page 24 ---	1-10
X	JOURNAL OF ANTIBIOTICS. vol. 41, no. 2, February 1988, TOKYO JP pages 220 - 225 T SAINO ET AL. 'protease-inhinbitory activities of leupeptin analogues' see table 1, examples 23 and 24 --- -/--	1-10

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
28 JANUARY 1993	12.02.9[illegible]
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	p. masturzo

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	JOURNAL OF MEDICINAL CHEMISTRY vol. 33, no. 1, January 1990, WASHINGTON US pages 86 - 93 R M MCCONNELL ET AL. 'new leupeptin analogues: syntehsis and inhibition data' see table 1 ---	1-10
X	CHEMICAL ABSTRACTS, vol. 111, no. 15, 9 October 1989, Columbus, Ohio, US; abstract no. 134761t, T. TANAKA ET AL. 'a process for preparation of peptides containing L-lysinal, useful as inhibitors of lysine-specific proteases' page 820 ;column RIGHT ; see abstract & JP,A,0 138 050 (WAKO PURE CHEMICAL INDUSTRIES) 8 February 1989 ---	1-10
X	CHEMICAL ABSTRACTS, vol. 110, no. 11, 13 March 1989, Columbus, Ohio, US; abstract no. 93555n, N SATO ET AL. 'manufacture of protease inhibitor MR-33-A with Streptomyces' page 560 ;column RIGHT ; see abstract & JP,A,6 339 898 (MARUISHI PHARMACEUTICAL CO., LTD.) 20 February 1988 ---	1-10
A	EP,A,0 313 969 (NITTO BOSEKI CO., LTD.) 3 May 1989 see the whole document ---	1-10
A	AGRICULTURAL AND BIOLOGICAL CHEMISTRY. vol. 49, no. 3, March 1985, TOKYO JP pages 799 - 805 K OGURA ET AL. 'purification and structure of a novel cysteine proteinase inhibitor, strepin P-1' see figure 2 --- -/--	1-10

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	CHEMICAL ABSTRACTS, vol. 96, no. 21, 24 May 1982, Columbus, Ohio, US; abstract no. 174592s, G BORIN ET AL. 'synthesis of leupeptins and inhinbition of proteinases. I. Inhibition of acrosin and trypsin' page 94 ;column LEFT ; & HOPPE-SEYLER`S Z. PHYSIOL. CHEM. vol. 362, no. 11, 1981, pages 1435 - 1445	1-10

International application No.

PCT/US 92/ 08901

INTERNATIONAL SEARCH REPORT

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: (1-5)*, (8-10)* *- incompletely
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Since the language of the claims abounds in imprecise and undefining terms (e.g. Trp residue may in no case be included in the wording of claim 1) the search has been limited to the subject matter as defined by the claims 6 and 7.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9208901
SA 66042

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 28/01/93

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
FR-A-2490632	26-03-82	JP-C- 1585234	31-10-90
		JP-B- 2000342	08-01-90
		JP-A- 57054157	31-03-82
		DE-A- 3137280	03-06-82
		GB-A,B 2086380	12-05-82
		US-A- 4401594	30-08-83
EP-A-0195212	24-09-86	AU-B- 600226	09-08-90
		AU-A- 5288186	07-08-86
		JP-A- 61183253	15-08-86
EP-A-0313969	03-05-89	JP-A- 1117900	10-05-89
		US-A- 4883863	28-11-89